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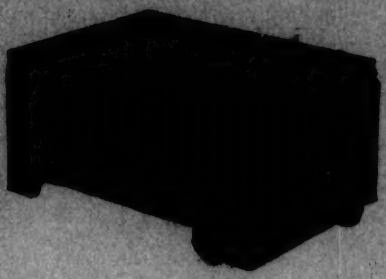

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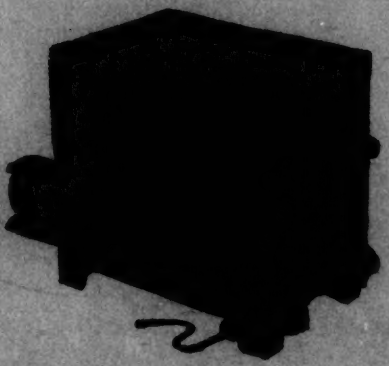

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THE CALCIUM CONTENT OF HUMAN BLOOD.

By HENRY LYMAN.

(From the Research Laboratory of the Huntington Memorial Hospital, and the Biochemical Laboratory of the Harvard Medical School, Boston.)

(Received for publication, March 29, 1917.)

The accompanying table gives the result of the analyses for calcium of fifty-four samples of human blood, determinations of the total non-coagulable nitrogen also being done on the pathological cases. It is hoped that these figures may help to establish a base line for further study of the metabolism of this metal. The chief point brought out seems to be that the calcium varies but little in a great majority of subjects. Even in cases of advanced uremia, where the total nitrogen was extremely high, the calcium was not increased. On the other hand, the hemophiliac and the case of purpura hemorrhagica were not lower than what we may regard as normal.

The average for the males, with the exception of No. 36, was 6.1 mg. of calcium per 100 cc. of blood; while that for females was 7.1, a difference sufficiently great to stimulate further investigation.

In No. 36 there seems to be no reason for the extremely low figure shown. Duplicate determinations were of course performed and there is no doubt that this represents the correct calcium content. Unfortunately, as the patient soon left the hospital, a second specimen could not be obtained. No. 8, on the other hand, was taken from a healthy young woman whose history gives us no clue to the explanation of the high result—9.6 mg. per 100 cc.

Determination of calcium was carried out according to the new method of the author,[1] while the total nitrogens were done by the direct Nesslerization process of Folin and Denis.[2] As it was more convenient to perform both analyses in the same filtrate, the following technique was employed:

[1] Lyman, H., *J. Biol. Chem.*, 1917, xxix, 169.

[2] Folin, O., and Denis, W., *J. Biol. Chem.*, 1916, xxvi, 473.

Blood was drawn into a 20 cc. paraffined pipette and run into a flask containing 55 cc. of distilled water. After agitating to lake the blood, 5 cc. of 80 per cent trichloroacetic acid were added, the whole well shaken, and allowed to stand for an hour or longer, as convenient. After filtering through calcium-free filter paper, 10 cc. of the filtrate were used for the calcium determinations and 5 cc. for the total nitrogens. The coagulation by trichloroacetic acid was first carefully checked by the phosphoric acid method of Folin and Denis and found to give identical results.

Not only does this procedure lessen the number of flasks and solutions to be carried about, but it also shortens the time of the nitrogen determinations, as there are only 5 cc. of fluid to boil off instead of 10 cc. Moreover, no pebble or bead need be used in the digestions as there is no bumping.

The specimens, with the exception of the normal cases, were obtained from the Massachusetts General Hospital, the Peter Bent Brigham Hospital, and the Huntington Memorial Hospital.

TABLE I.

Calcium and Total Non-Coagulable Nitrogen per 100 Cc. of Human Blood.

Case.	Sex.	Diagnosis.	Ca	N
			mg.	*mg.*
1	M.	Normal.	6.1	
2	"	"	6.2	
3	"	Chronic infectious arthritis.	5.9	
4	"	Normal.	6.1	
5	"	"	6.1	
6	F.	"	7.2	
7	"	Acromegaly.	7.3	
8	"	Normal.	9.6	
9	"	"	7.7	
10	M.	"	6.1	
11	"	"	6.4	
12	"	"	6.7	
13	"	"	5.2	
14	"	Carcinoma of jaw.	6.4	
15	"	" " larynx.	6.0	
16	"	" " eyelid.	6.4	
17	"	Hemophiliac.	6.0	39.4
18	"	Purpura hemorrhagica.	6.4	37.0
19	"	Myelogenous leukemia.	6.4	61.8
20	"	Cardiac.	6.1	52.0
21	"	Nephritis.	6.0	56.6
22	"	Hyperthyroid.	6.9	41.0

TABLE I—*Concluded.*

Case.	Sex.	Diagnosis.	Ca	N
			mg.	*mg.*
23	M.	Diabetic (16 yrs.).	4.8	32.6
24	"	Syphilis.	6.5	35.6
25	"	Amebic dysentery.	4.2	32.0
26	"	Tumor of orbit, malignant (?).	5.3	41.0
27	"	Myelogenous leukemia.	6.2	47.4
28	"	Gout.	6.0	44.8
29	"	Syphilis.	5.5	36.2
30	"	Gout and uremia.	5.7	76.8
31	"	Pleurisy with effusion.	5.5	44.4
32	"	Nephritis.	6.6	49.2
33	"	Tuberculosis.	6.1	36.4
34	"	Cardiac, in extremis.	6.8	98.8
35	"	Cardiorenal with asthma.	6.4	50.0
36	"	Gout (?), congenital heart, hemoglobin 160 per cent.	2.8	37.8
37	"	Hyperthyroid.	6.4	32.0
38	F.	Chronic nephritis, uremia, clonic twitching.	5.2	134.4
39	"	Carcinoma (?) of stomach.	7.3	35.8
40	"	Diarrhea.	6.4	30.2
41	"	Multiple neuritis.	6.1	44.8
42	"	Hyperthyroid.	6.4	31.0
43	"	Diabetes.	6.7	29.2
44	"	Acute nephritis.	7.6	39.2
45	M.	Myelogenous leukemia.	6.3	45.6
46	"	Carcinoma of pharynx.	6.6	35.2
47	"	" " eyelid.	6.7	38.2
48	"	" " tongue.	6.9	31.0
49	"	Sarcoma of prostate.	6.5	30.8
50	F.	Carcinoma of rectum.	7.8	32.4
51	"	" " cervix.	6.8	39.2
52	"	" " "	7.4	25.2
53	M.	Sarcoma of glands of neck.	6.5	34.0
54	"	Normal.	5.5	

THE MECHANISM OF CYTOLYSIS IN ECHINODERM EGGS.

Second Paper.

By A. R. MOORE.

(From the Physiological Laboratory of Rutgers College, New Brunswick, N. J., and the Marine Biological Laboratory, Woods Hole, Mass.)

(Received for publication, March 16, 1917.)

In the previous paper[1] it has been shown that the cytolysis reaction in the case of the eggs of the sea urchin, *Strongylocentrotus purpuratus*, depends upon a chemical reaction of the first order. In these experiments the material was homogeneous; *viz.*, in each experiment the eggs were from one individual and were all mature and unfertilized. If one attempts to repeat the experiment with the eggs of starfish, the results fail to show a uniform progress of the reaction. This is apparently due to the fact that the starfish eggs from a given individual are not homogeneous. If a lot of these eggs are allowed to stand in the laboratory for a day and are then examined, very often sufficient change has occurred to render it possible to group them into three classes by optical and experimental means; *viz.*, (1) immature eggs with irregular outline and large nuclei, incapable of fertilization; (2) recently mature eggs, regular spheres, transparent yellowish brown in color, which may be fertilized and are therefore alive; (3) old mature eggs, black-brown in color, which cannot be fertilized and are therefore dead. These differences have been shown to be dependent upon oxidations, and the changes of maturation and disintegration can be inhibited by keeping the eggs in an atmosphere of hydrogen or by adding small quantities of KCN to the solution containing them.[2] Therefore, it seems reasonable to suppose that the optical and vital differences described are

[1] Moore, A. R., *J. Biol. Chem.*, 1916–17, xxviii, 475.

[2] Loeb, J., Artificial Parthenogenesis, Chicago, 1913, 27.

expressions of underlying chemical differences, and that these chemical differences are dependent upon the degree to which certain oxidation reactions have developed; in other words, upon the respective ages of the cells. These chemical differences due to age should become apparent in the cytolysis reaction; *i.e.*, eggs having the least natural resistance should cytolyze first, those having greatest natural resistance cytolyze last, and those of average resistance, comprising the great majority of the cells, cytolyze in approximately half the time. A reaction of this type does not progress at a constant rate, like a chemical reaction depending upon the fortuitous interaction of ions or molecules, but at the outset has a low velocity, when half completed has its maximum velocity, and in the last phase has a low velocity. Arrhenius[3] has suggested a simple method for determining whether reactions of cells proceed at the uniform rate of a chemical reaction or are dependent upon the age and natural resistance of the units taking part in the process. According to this method, if the $\log e\ N$ (N, number of cells surviving at the end of time, t) is plotted as ordinates against the time, t, as abscissæ, the graph is a straight line in the case of a chemical reaction, but a double curve graph in case the reaction depends upon the natural resistance of the cells. The following experiments show that the cytolysis reaction of starfish eggs follows the second type of reaction; *i.e.*, is dependent upon the specific chemical condition of each egg, which differs significantly with the age of the egg.

The experiments were carried out in the way previously described.[4] The material used consisted of the unfertilized eggs of the starfish, *Asterias forbesii*. After the initial lowering of the temperature to the extent of 0.2–0.3°C., due to the introduction of the charge of eggs into the cytolyzing chamber, the bath was kept within 0.1°C. of the temperature required.

The experiments recorded in Table I show the percentages of eggs remaining uncytolyzed at the end of each time interval. It is evident that the percentage of eggs cytolyzed each minute is not a constant percentage of the number remaining uncytolyzed. The graphs derived from the experimental data show that the

[3] Arrhenius, S., Quantitative Laws in Biological Chemistry, London 1915, 78.

[4] Moore, *Quart. J. Exp. Physiol.*, 1910, iii, 257.

TABLE I.

Time.	N	$\log_e N$.
	Experiment 1. $T = 39°$.	
min.		
1	83	4.41
4	53	3.97
6	33	3.49
8	10	2.30
10	6	1.79
	Experiment 2. $T = 38.7°$.	
1	77	4.34
2	52	3.95
4	47	3.85
6	18	2.89
8	16	2.77
	Experiment 3. $T = 39°$.	
1	93	4.53
2	87	4.46
4	80	4.38
6	66	4.18
8	14	2.64
10	10	2.30
	Experiment 4. $T = 39°$.	
1	94	4.54
2	82	4.40
4	63	4.14
6.25	15	2.70
8	7	1.94
10	4	1.38
	Experiment 5. $T = 39°$.	
1	100	4.60
2	88	4.47
4	65	4.17
6	45	3.80
8	18	2.89
10	5	1.61
	Experiment 6. $T = 39°$.	
1	78	4.35
2	71	4.26
4	36	3.58
6	22	3.09
8	20	2.99

reaction velocity is slow at first, most rapid when half completed, and slow again when the reaction nears completion.[5] In some cases the third phase does not appear. From these results it must be concluded that the rate of cytolysis of starfish eggs, *i.e.*, the time necessary at the given temperature for an egg to cytolyze, depends upon the chemical state of the egg, which in turn is determined by the age of the egg or the extent to which certain oxidations have taken place in it (Fig. 1).

The Cytolytic Effect of Treatment with the Alkaline Earth Chlorides.

It has been shown that treatment with an isosmotic solution of $SrCl_2$ greatly increases the rate of hot water cytolysis of the eggs of the sea urchin, *Strongylocentrotus purpuratus*.[6] In fact if the eggs of this form are allowed to remain in an isosmotic solution of $SrCl_2$ at room temperature, they form clear blebs at their peripheries and ultimately cytolyze. Solutions of $MgCl_2$, $CaCl_2$, and $BaCl_2$ bring about similar results but differ in certain characteristic ways in their action. Thus, in the solution of $MgCl_2$ the eggs swell and become clear; in the $CaCl_2$ they become clear with little if any swelling. In $BaCl_2$, as a rule, the cytoplasm remains optically unchanged, but a large bleb develops at the periphery, often equalling the egg in diameter; occasionally shadows form in this solution. KCl does not inhibit the cytolytic action of $MgCl_2$ and $CaCl_2$, nor are the two latter named salts mutually antagonistic. NaCl, however, when present in the ratio of 10 cc. of the M/2 solution to 90 cc. of a 3/8 M $CaCl_2$ or of a 3/8 M $MgCl_2$ solution completely inhibits the formation of blebs or of shadows.

In order to determine whether the chlorides of Mg, Ca, Sr, and Ba comparably affect the eggs of the starfish, *Asterias forbesii*, two types of experiments were carried out. First, the eggs were allowed to remain in the pure solutions of the chlorides of 3/8 M concentration for 12 to 24 hours. No definite cytolytic effects were observed. Second, the eggs, after being washed twice in the pure salt solution, were kept for 10 minutes in a third por-

[5] The writer is aware that this also describes in a general way the course of an autocatalytic monomolecular reaction, but if one tries to fit the data here given to the equation for such a reaction, it will be seen that the reaction proceeds much too rapidly at the outset for such an hypothesis to be applicable.

[6] Moore, *Arch. Entwcklngsmechn. Organ.*, 1913, xxxvii, 433.

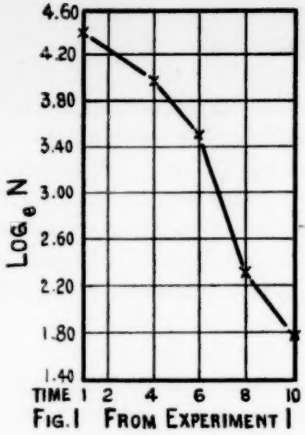

FIG. 1 FROM EXPERIMENT 1

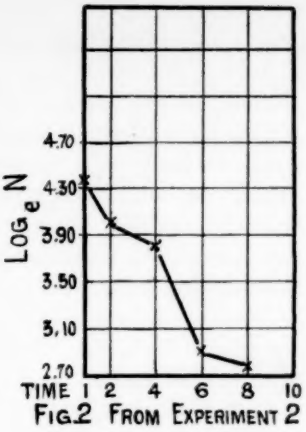

FIG. 2 FROM EXPERIMENT 2

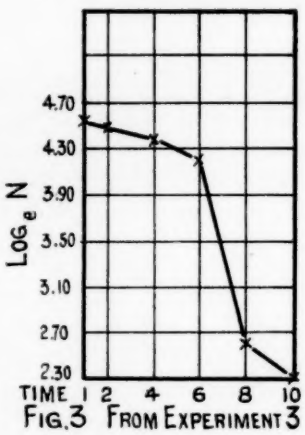

FIG. 3 FROM EXPERIMENT 3

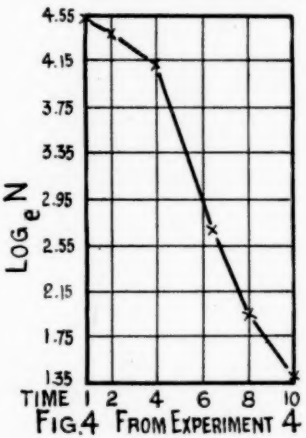

FIG. 4 FROM EXPERIMENT 4

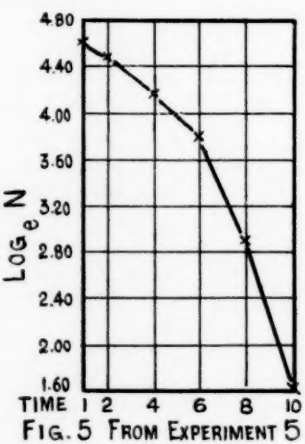

FIG. 5 FROM EXPERIMENT 5

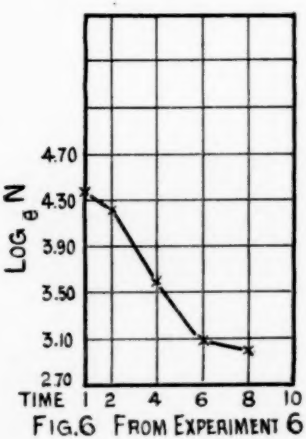

FIG. 6 FROM EXPERIMENT 6

FIG. 1.

tion of the solution and were then put into a cylinder of heated sea water kept at a constant temperature. The percentages of eggs cytolyzed were determined in the usual way. A parallel experiment with the untreated eggs of the same female was always run as a control. The experiments showed that in the eggs of the starfish the chlorides of Mg, Ca, Sr, and Ba not only fail to increase the rate of cytolysis but frequently retard it. The experiments in Table II illustrate this point. Since, in the case of sea urchin eggs, the chlorides of the alkaline earths exert a cytolytic action, the results of the experiments with starfish eggs indicate characteristic differences in the chemical constitution of the eggs of the two genera with respect to the phenomenon of cytolysis.

TABLE II.

Time.	Normal eggs cytolyzed.	Treated eggs cytolyzed.
	Experiment 1. $T = 39°$. $MgCl_2$.	
min.	*per cent*	*per cent*
2	12	5
4	35	
6	55	37
8	82	48
10	95	100
	Experiment 2. $T = 39°$. $CaCl_2$.	
	A.	
1	5	4
2	22	18
4	67	60
6	70	62
8	95	72
10	99	90
	B.	
1	100	5
2		20
4		80
6		90
8		95

TABLE II—*Concluded.*

Time	Normal eggs cytolyzed.	Treated eggs cytolyzed.
Experiment 3. $T = 39°$. $SrCl_2$.		
A.		
1	1	0
2	2	0
4	2	2
6	40	
8		20
10	80	25
15	95	50
B.		
1	23	22
2	48	29
4	53	64
6	82	78
8	84	80
Experiment 4. $T = 39°$. $BaCl_2$.		
1	9	1
2	30	
4	48	5
6	80	
8	98	
10	100	10

THE SUPPLEMENTARY DIETARY RELATIONSHIP BETWEEN LEAF AND SEED AS CONTRASTED WITH COMBINATIONS OF SEED WITH SEED.*

By E. V. McCOLLUM, N. SIMMONDS, and W. PITZ.

(From the Laboratory of Agricultural Chemistry of the Wisconsin Experiment Station, Madison.)

(Received for publication, March 26, 1917.)

In previous papers we have described the nature of the dietary deficiencies of some of the most important seeds which serve as food for man and animals; *viz.*, wheat,[1] maize,[2] rice[3] and oat kernels,[4] and the white bean.[5] With the exception of polished rice and the bean these seeds closely resemble each other in their dietary properties: (*a*) the proteins being of relatively poor quality in that certain essential amino-acids are furnished by them in amounts below the optimum; (*b*) the content and composition of the inorganic portion of each seed are of a character which cannot induce normal growth, and must be corrected by certain salt additions; and (*c*) the content of the fat-soluble A is inadequate to supply the needs of the growing animal over a prolonged period. Unpolished rice resembles closely the other grains mentioned above, but polished rice, which has lost both its bran layer and its germ in the process of polishing, lacks, in addition to the deficiencies mentioned above, the second unidentified dietary

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[1] Hart, E. B., McCollum, E. V., Steenbock, H., and Humphrey, G. C., *Wisconsin Agric. Exp. Station Research Bull. 17*, 1911. Hart and McCollum, *J. Biol. Chem.*, 1914, xix, 373. Hart, E. B., Miller, W. S., and McCollum, E. V., *ibid.*, 1916, xxv, 239. McCollum, E. V., Simmonds, N., and Pitz, W., *ibid.*, 1916–17, xxviii, 211.

[2] Hart and McCollum, *J. Biol. Chem.*, 1914, xix, 373. McCollum, Simmonds, and Pitz, *ibid.*, 1916–17, xxviii, 153.

[3] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxiii, 181.

[4] McCollum, Simmonds, and Pitz, *J. Biol. Chem.*, 1917, xxix, 341.

[5] McCollum, Simmonds, and Pitz, *J. Biol. Chem.*, 1917, xxix, 521.

factor, water-soluble B. The ordinary bean differs from the other seeds mentioned particularly in the poor quality of its proteins. These contain but little of some one or more essential amino-acids and it becomes imperative that beans should be combined with such other natural foods as furnish proteins which supplement these deficiencies and therefore enhance their value.

In the present paper we present certain further data which point clearly to the general lines on which successful nutrition is to be attained, when the diet is derived solely from vegetable sources. The discussion will be limited to the results obtained with combinations of seeds from several sources, and of seeds with the alfalfa leaf. We reserve for future discussion the possibilities of obtaining normal nutrition during growth and reproduction with simple combinations of tubers with grains and of fruit juices with grains.

Our experience over a decade with both rats and swine has convinced us that the nutritive requirements for these two species are essentially the same. Neither species can grow satisfactorily when restricted to one of the cereal grains, and both respond in about the same way with growth and reproduction, to specific modifications of the diet when thus restricted as to source.

It was somewhat of a surprise to us to find that we were not able to make up a ration derived solely from the seeds of plants which would serve to support normal nutrition during the growing period, even though two to five seeds of widely different varieties were employed. Results of a very different character are secured when simple combinations of leaf and seed are fed as monotonous diets. The interpretation of the curves of growth in the charts presented in this paper bring out the following points:

1. We have failed to secure any appreciable amount of growth with the following mixtures of seeds when salt-free water was supplied; (*a*) maize 90, flaxseed oil meal 10, Chart 1; (*b*) wheat 20, maize 20, rolled oats 20, hemp seed 20, millet seed 20, Chart 4; (*c*) wheat 25, maize 25, rolled oats 25, hemp seed 25, Chart 3; (*d*) wheat 33, maize 33, rolled oats 33, Chart 2. These rations represent two distinct types: (*a*) and (*b*) serve to promote good growth if suitable inorganic salt additions are made, but not otherwise (Charts 1 and 4). This means that the mixtures sup-

ply enough of both the unidentified dietary factors fat-soluble A and water-soluble B, as well as the long recognized dietary factors, proteins of adequate quality, and assimilable energy as carbohydrate and fat. When considered in the light of our former experience with maize, wheat, and oats, all of which have been shown to carry an inadequate amount of the fat-soluble A, it becomes evident that the flaxseed meal and millet seed differ from maize, wheat, and oats in containing a much greater amount of this dietary factor. This conclusion is further supported by the records of rats fed (*c*) (Chart 3). After a period of suspended growth they failed to respond so well with growth when a salt mixture was added as did those whose diets contained either flaxseed or millet seed (Charts 1 and 4). Hemp seed appears to be somewhat richer in the fat-soluble A than are wheat, oats, and maize. Ration (*d*) does not support growth, and Period 2 in the record of Lot 714 (Chart 2) shows clearly that it is not solely the shortage of the fat-soluble A which is responsible. Our experimental work does not yet include the feeding of wheat, oat, and maize mixtures with salts alone or purified protein alone, but in view of the remarkable degree to which salt additions induce growth on mixtures of these seeds with hemp seed and millet seed (Charts 3 and 4) there is little doubt that this is one of the limiting factors in the case of Lot 714. In other words, with wheat, oats, and maize in equal proportions the data available indicate that both salts and fat-soluble A must be added before growth can take place.

The growth records shown in Charts 1 to 4 make possible the generalization that *it is difficult if not impossible to obtain even a moderate amount of growth over an extended period on a diet restricted to the seeds of plants.* It is evident that satisfactory protein mixtures can be had from seed mixtures, and from the results shown in Charts 1 and 4 it is further evident that certain seeds as flaxseed and millet seed contain the fat-soluble A in fairly liberal amounts. Since the water-soluble B is everywhere abundant in the seeds the cause of failure to secure growth on seed mixtures is seen to lie in the amount and character of the inorganic moiety.[6] An in-

[6] Unpublished data in our records indicate that the mixed proteins of wheat, oat, and maize fed at this plane of protein intake (10 to 11 per cent) are of satisfactory character to support fairly good growth when the rations are not otherwise deficient.

spection of the literature relating to the content of inorganic elements in various classes of natural foods as determined in recent times by satisfactory methods reveals at a glance the supplementary relationships among the different classes of vegetable foods in inorganic elements. Of the seven most important seeds from the standpoint of human nutrition and animal production, only cottonseed and flaxseed contain a high total inorganic content and in both cases the ash is very poor in three important elements, sodium, calcium, and chlorine. Since a pronounced deficiency of these elements is characteristic of all other seeds as well, no combinations of seeds will supply these elements in satisfactory amounts. Since spring and well waters in certain districts contain both sodium and calcium in greatly varying amounts it would be expected that animals may secure the necessary inorganic supplements through the drinking water in some localities which would enable them to grow on a ration restricted to seeds, whereas in other parts of the world they could not do so.

But few reliable data are available showing the variation of the inorganic content of seeds as influenced by the character of the soil. It is a well known principle of physiology that plants have but limited selective capacity with reference to minerals in solution in the soil water. Forbes and his coworkers[7] made an extensive investigation of the inorganic elements of blue grass from different types of soils and found that at the same stage of growth samples from different localities showed variation between 4.80 and 8.66 per cent of ash, calcium varied between 0.135 and 0.424 per cent, potassium between 1.41 and 2.85 per cent, and phosphorus between 0.164 and 0.403 per cent.

Ames[8] states that the composition of wheat (grain) grown on the unfertilized plots of two soils, containing different amounts of phosphorus, potassium, and nitrogen is in accordance with the composition of these soils, and that the proportion of these elements in the wheat plant is increased by the addition of these elements to the soil. Under natural conditions distinct differences will doubtless be observed in the capacity to grow of animals

[7] Forbes, E. B., Whittier, A. C., and Collison, R. C., *Ohio Agric. Exp. Station Bull. 22*, 1910.

[8] Ames, J. W., *Ohio Agric. Exp. Station Bull. 221*, 1910. Ames, J. W., Boltz, G. E., and Stenius, J. A., *ibid.*, *243*, 1912.

*Inorganic Constituents of Vegetable Foodstuffs.**

	Ash.	K	Na	Ca	Mg	P	Cl
			Leaf.				
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Alfalfa hay	7.38	1.64	0.1	2.14	0.22	0.34	0.29
Rape green	8.08	2.23	0.23	1.26	0.18	0.40	0.61
Red clover	6.86	1.84	0.10	1.71	0.45	0.29	0.26
Rhubarb	14.49	7.14	0.55	1.04	—	0.93	0.77
Spinach	16.48	2.27	4.32	1.40	0.63	0.74	1.04
Cabbage heart	10.85	2.51	0.80	1.66	0.23	0.71	0.85
			Seed.				
Rice hulled	0.39	0.07	0.016	0.009	0.026	0.09	0.0004
Maize	1.55	0.32	0.05	0.01	0.12	0.33	0.045
Wheat	2.08	0.50	0.02	0.04	0.14	0.40	0.06
Oat meal	1.83	0.36	0.06	0.01	0.09	0.38	0.10
Beans	3.63	1.25	0.03	0.13	0.16	0.61	0.06
Cottonseed meal	7.48	1.85	—	0.23	0.68	1.5	0.003
Linseed meal	5.84	1.18	0.07	0.35	0.55	0.80	0.04
			Tuber, root.				
Potato	3.79	1.90	0.08	0.07	0.11	0.28	0.13
Sweet potato	3.07	1.28	0.15	0.22	0.06	0.14	0.39
Beet	5.97	0.84	2.16	0.25	0.01	0.25	0.29
Turnip	8.01	3.02	0.58	0.60	0.18	0.44	0.40
			Fruit.				
Oranges	3.08	0.93	0.31	0.54	0.15	0.15	0.07
Apples	1.44	0.43	0.28	0.04	0.07	0.08	—
Plums	2.08	1.20	0.03	0.06	0.06	0.12	0.007
Raisins	2.86	1.10	0.16	0.10	0.10	0.22	0.14
Figs	2.92	1.35	0.05	0.23	0.10	0.16	0.06

* Selected from the paper by E. B. Forbes, *Ohio Agric. Exp. Station Bull. 207*, 1909.

confined to a diet of seeds but permitted access to the soil and furnished ground or drainage water, because of the variable factors mentioned above. It is a matter of great importance, however, that we should fully appreciate the peculiar dietary properties of the different classes of vegetable foods and bear in mind

the importance of making suitable combinations which will approximate the optimum in content and proportions among the inorganic elements.

The degree to which the inorganic content of certain mixtures of seed and leaf supports growth will be seen from an inspection of Charts 5 to 14. It should be kept in mind, however, that a complete explanation is not available of the cause of the relatively poor nutrition of many of the animals fed the rations described in this paper. Not until those food mixtures which fail to nourish young animals properly are fed supplemented with certain single and multiple additions of purified food substances can such explanations be made. Where little or no growth was observed certain dietary factors other than protein, inorganic constituents, or unidentified factors A and B may be the cause. These are to be excluded by the results of suitably planned experiments.

A second generalization of fundamental importance in nutrition is now possible.[9] *The leaf is distinctly different from the seed in its dietary properties in two respects: its total inorganic content is very high, and it is especially rich in both sodium and calcium, both of which are deficient in the seeds generally.* In addition the leaf of the plant is several times richer in fat-soluble A than are the wheat, oat, and maize kernels. Certain seeds approximate the value of the leaf in this substance. Hemp seed is distinctly better than those just named, but flaxseed and millet seed are still richer than hemp seed and may readily be incorporated in the diet in amount sufficient to meet the needs of an animal for the fat-soluble A during growth (Charts 1 and 4).

It is interesting to note that the content of the fat-soluble A is highest in those seeds which are smallest (flax and millet). It seems not unlikely that this may in some measure be related to the relatively large proportion of germ as compared with endosperm in such seeds. The endosperm is in great part to be likened to a mixture of purified proteins, carbohydrates, and fats, while the germ is relatively rich in functioning plant cells as well as fats, carbohydrates, etc. The great differences in the amount of stored food material contained in the germ in various seeds ren-

[9] McCollum, Simmonds, and Pitz, *Am. J. Physiol.*, 1916, xli, 333.

der it impossible to make such a comparison very accurate. It seems reasonable to suppose that the differences in the dietary properties of the entire seed as contrasted with the embryo,[10] and of polished rice as contrasted with hulled rice, may lie in part in the association of the unidentified dietary A and B with the functioning cell. The same reasoning may be applied to account for the exceptional richness of the leaf in these two dietary principles. The leaf is the seat of great synthetic activity and consists relatively of a large amount of functioning cells associated with those substances which may be considered as the equivalent of purified protein, carbohydrate, and fats.

We have pointed out in a previous paper[11] that the isolated plant fats as obtained either by extraction with a fat solvent or by hot pressing do not contain the fat-soluble A. Suitably planned feeding experiments have shown that it is the fat-free residue of the seed which still contains the content of this substance which was originally present in the seed. We have demonstrated this only in the case of the maize kernel[2, 11] and the flaxseed (Chart 1, Lot 722), but the experiments of Richardson and Green[12] indicate that cottonseed flour still contains a fair amount of this dietary factor. We have shown that isolated cottonseed oil is without the peculiar growth-promoting property which it would show if it contained this substance.[11]

[10] McCollum, Simmonds, and Pitz, *J. Biol. Chem.*, 1916, xxv, 105.
[11] McCollum, Simmonds, and Pitz, *Am. J. Physiol.*, 1916, xli, 361.
[12] Richardson, A. E., and Green, H. S., *J. Biol. Chem.*, 1916, xxv, 307.

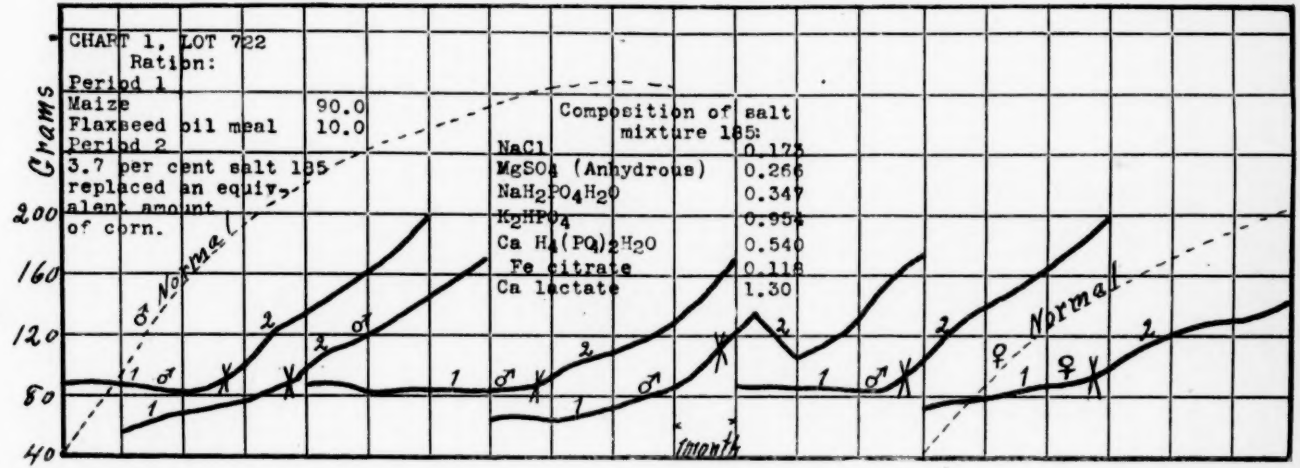

CHART 1. Lot 722 illustrates the failure of young rats to grow on a diet consisting of 90 per cent ground maize and 10 per cent flaxseed oil meal. The sole reason for the stunting lay in the unsatisfactory character of the inorganic content of the food mixture. After 3 to 4 months of suspended growth the animals began to increase in body weight at nearly the normal rate when 3.7 per cent of a suitably constituted salt mixture was added. The fact that growth took place indicates that the protein mixture was of satisfactory character.[13] Salt-free water was supplied in all the experiments.

[13] McCollum, *J. Biol. Chem.*, 1914, xix, 323.

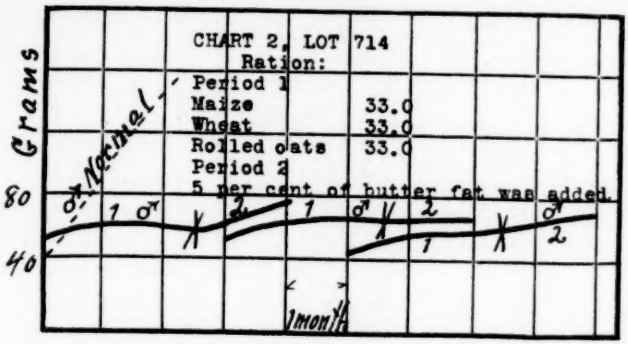

CHART 2. Lot 714 illustrates the failure of animals to grow when given a mixture of wheat, oats, and maize in equal proportions with distilled water. Two factors operate in contributing to the inadequacy of this diet for growth; *viz.*, there is an unsatisfactory inorganic content and a shortage of the fat-soluble A. In Period 2 the latter factor was added in butter fat, but there could be no response with growth without the deficiencies of the inorganic portion of the food mixture being made good. The mineral deficiencies of each of these grains have been demonstrated elsewhere.[1, 2, 4]

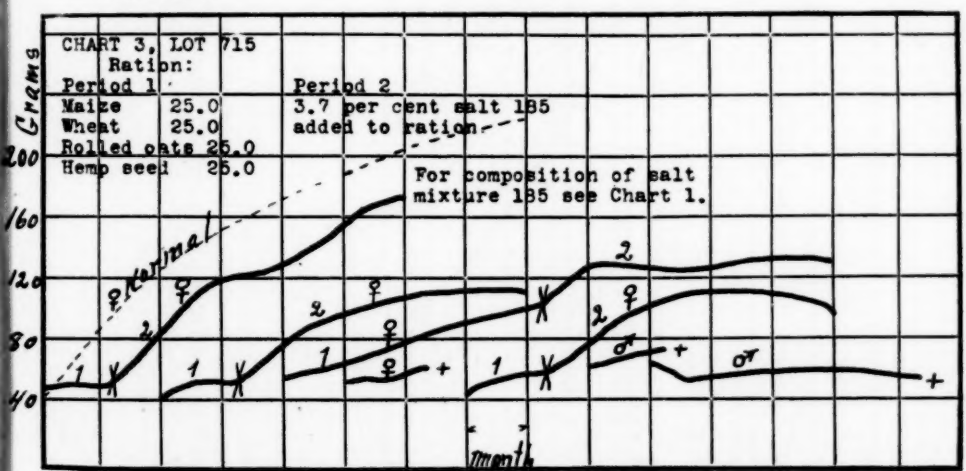

CHART 3. Lot 715. Practically no growth can be secured with even four seeds, as the above records show. There was but a fair response with growth when a suitable salt mixture was added after a period of suspension. The reason for this lies in the low content of the unidentified fat-soluble A in these seeds. Hemp seed is considerably richer than the other seeds in the mixture in this substance so the rats were enabled to grow at a rate below the normal. If both salt and butter fat additions had been made simultaneously, growth would have been much more rapid.

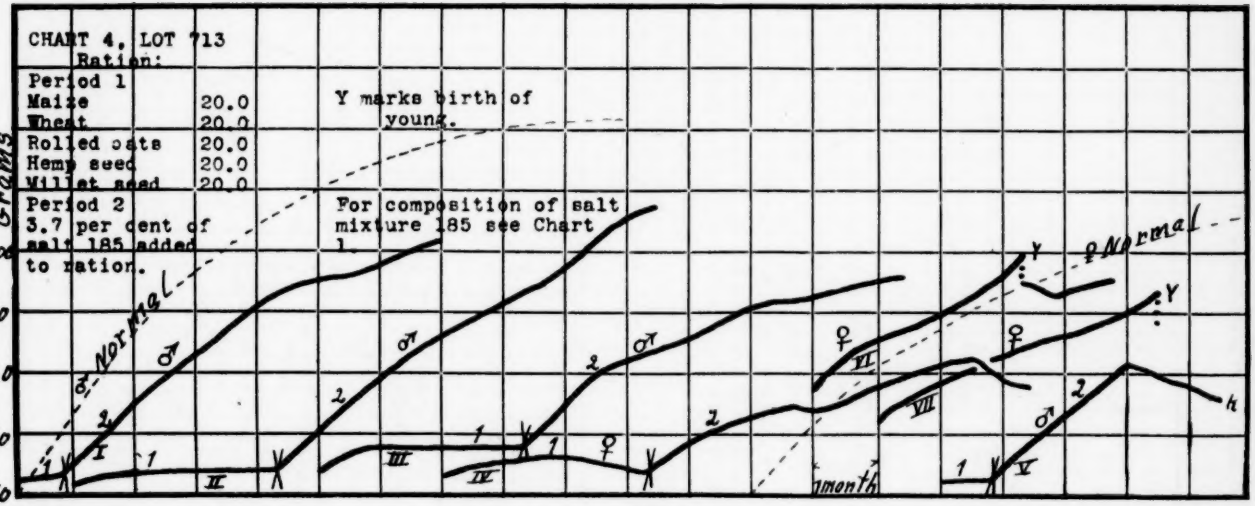

CHART 4. Lot 713. Even five seeds in wholesome condition when mixed in equal proportions cannot support growth when fed without the possibility of an accessory salt supply, as might happen if certain well or spring waters were taken. The sole reason for the failure of these animals to grow is found in the fact that the seeds generally furnish but an inadequate amount of the inorganic elements essential for normal metabolism, and all are especially poor in the elements sodium and calcium. In Period 2 the addition of 3.7 per cent of Salt Mixture 185 induced a prompt resumption of growth. The resumption of growth was possible because millet seed like flaxseed contains a fairly liberal amount of the fat-soluble A. Hemp seed contains more than either wheat, oats, or maize, all of which are distinctly poor in this dietary factor. Compare with Charts 2 and 3. Rat VI, which was fed this seed mixture with salt additions from the beginning, has successfully weaned five young. These weighed collectively 412 gm. at 72 days of age.

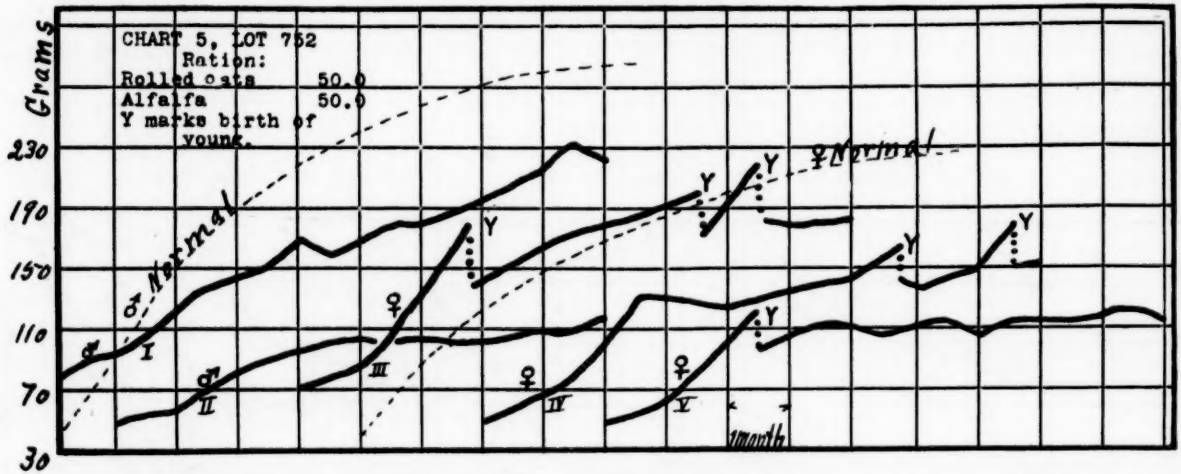

CHART 5. Lot 752. These curves illustrate the remarkable difference in the growth-promoting properties of a food mixture of the oat kernel with the alfalfa leaf in equal proportions as contrasted with the several mixtures of seeds alone described in Charts 1 to 4. Of the five litters of young born (thirty young) three young were successfully weaned. Four litters were eaten by the mothers within a few days. Rat III kept her last litter of six to the age of 14 days. They were then scaly and scrawny and weighed but 12.4 gm. each. Three have reached the age of 41 days and weigh but 37 gm. each. The normal for this age is about 60 gm. On becoming able to eat of the mother's ration they became bright and active. The alfalfa content of this ration is above the optimum (Chart 6).

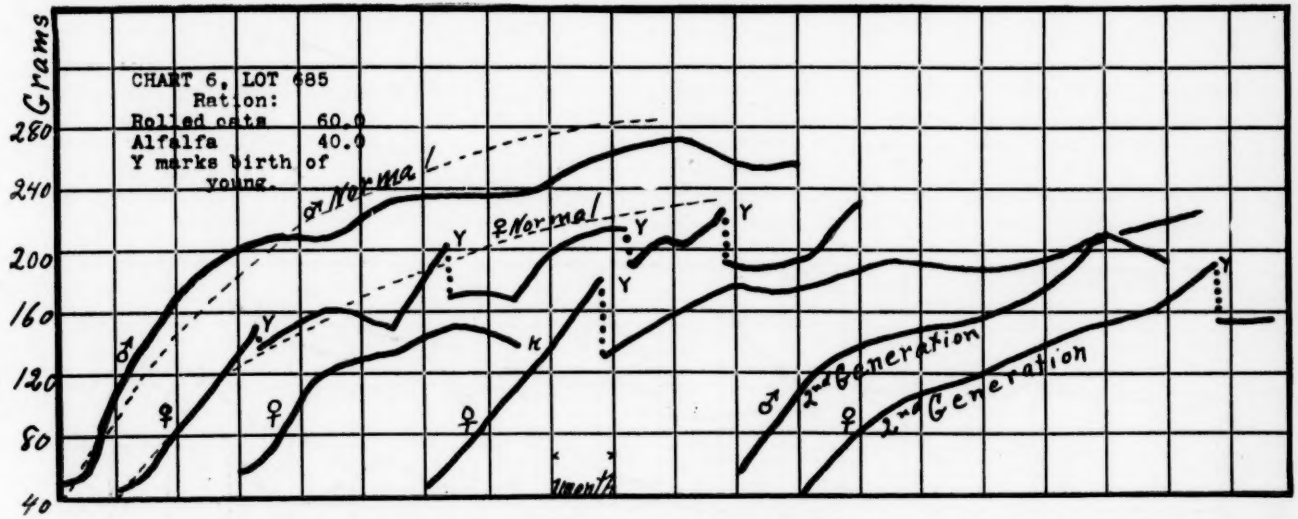

CHART 6. Lot 685. Rolled oats 60 and alfalfa leaf 40 per cent make a surprisingly good ration for the rat, both for growth and for rearing of the young. This ration is much better than is a mixture of oats and alfalfa leaf in equal amounts (Chart 5). All the records of our numerous trials of alfalfa with other food materials indicate that it should never contribute more than 40 per cent of the food mixture. Oats and alfalfa form a better monotonous ration than does alfalfa with any other seed we have studied (compare Charts 5 to 14). The proportions furnished to these animals, 40 of alfalfa to 60 of oats, are the most satisfactory for the promotion of well-being. Of the six litters (thirty-three young) sixteen young were successfully weaned but were somewhat undersized. Two of these have grown at somewhat below the normal rate to the age of 6 months. The nutrition of the animals in Lot 685 is below the optimum, but this can be seen only in the high mortality of the young, and in the fact that there is a tendency for the rats to remain undersized. This is especially true of the second generation confined to this ration.

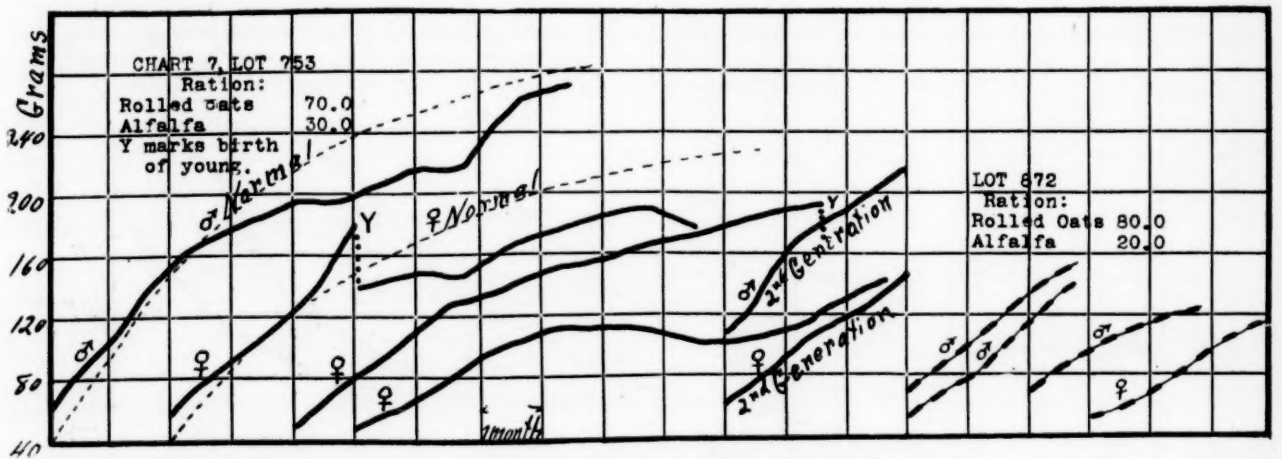

CHART 7. Lot 753. This chart confirms the statement made in discussing Chart 6, that rolled oats and alfalfa leaf in the proportions 60 to 40 form the most satisfactory mixture of these two substances (compare Charts 5 to 8). With a mixture of oats 70 and alfalfa 30 nearly complete growth was secured in half the animals, the rest suffering partial stunting. Of three females in this group one has produced no young and the other two but single litters of six and three. Five of these young were successfully weaned.

Lot 872 shows the rate of growth during the first 3 months on a diet of 80 per cent of oats and 20 of alfalfa.

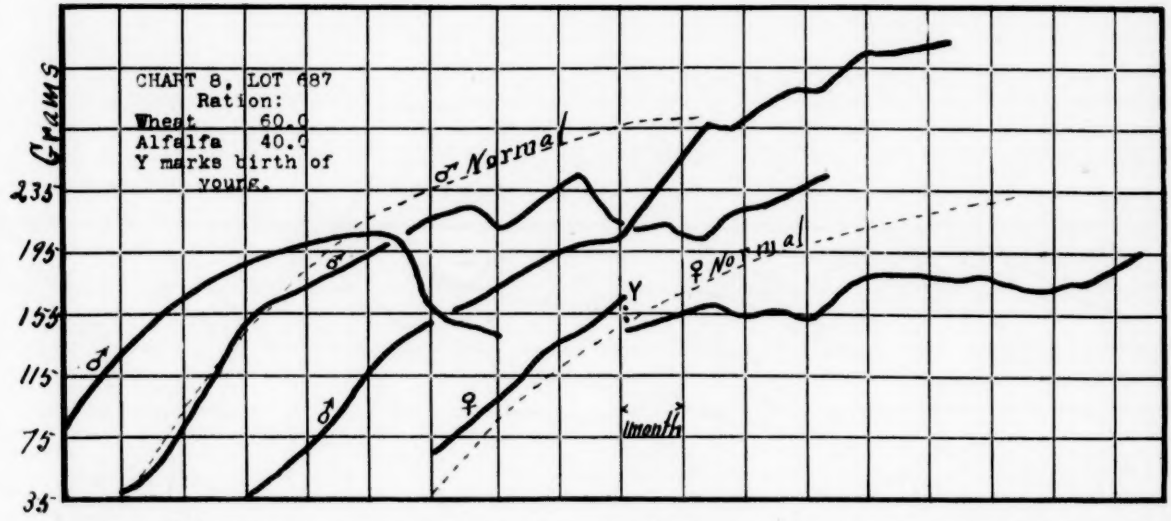

CHART 8. Lot 687 illustrates the irregularity in the course of the growth curves, but all approached the normal curves very closely. This lot contained but one female and she has produced but one litter of young, all dead when born. It is evident that 60 of wheat with 40 of alfalfa is a distinctly inferior ration as compared with the same proportions of oats and alfalfa (Chart 6).

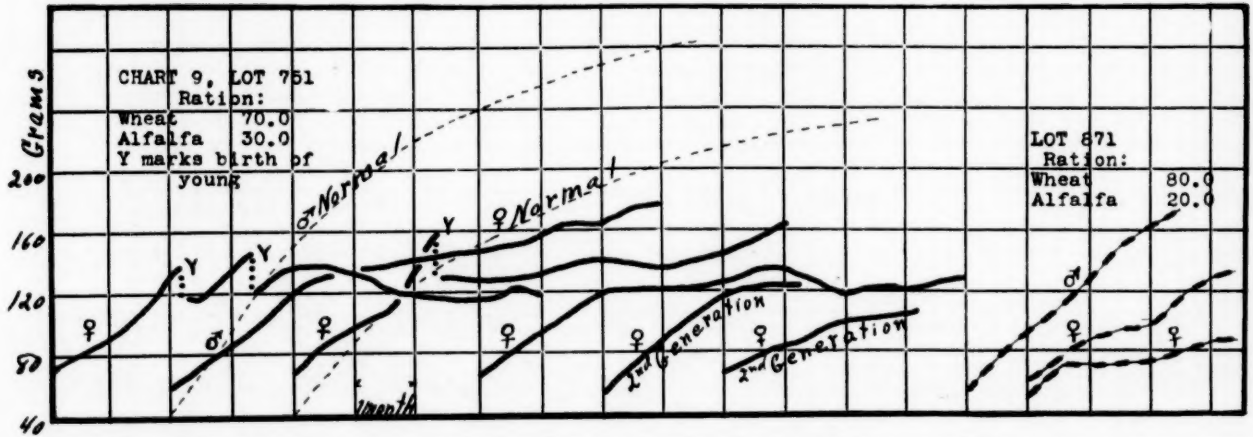

CHART 9. Lot 751 shows the pronounced stunting suffered by rats fed 70 per cent of wheat and 30 per cent of alfalfa. One of the three litters of young born was successfully weaned. These young are failing to grow on this diet. The inferiority of wheat and alfalfa combinations as contrasted with similar mixtures of oats and alfalfa for growth is very striking (compare with Chart 7).

Lot 871. Three of the four rats are markedly stunted and the fourth is growing at a rate slower than the normal on wheat 80 and alfalfa 20 per cent. It will be necessary to make a special series of feeding trials with each of these wheat and alfalfa mixtures supplemented with single and multiple additions of purified foodstuffs in order to learn the specific reason for their unsatisfactory nutrition.

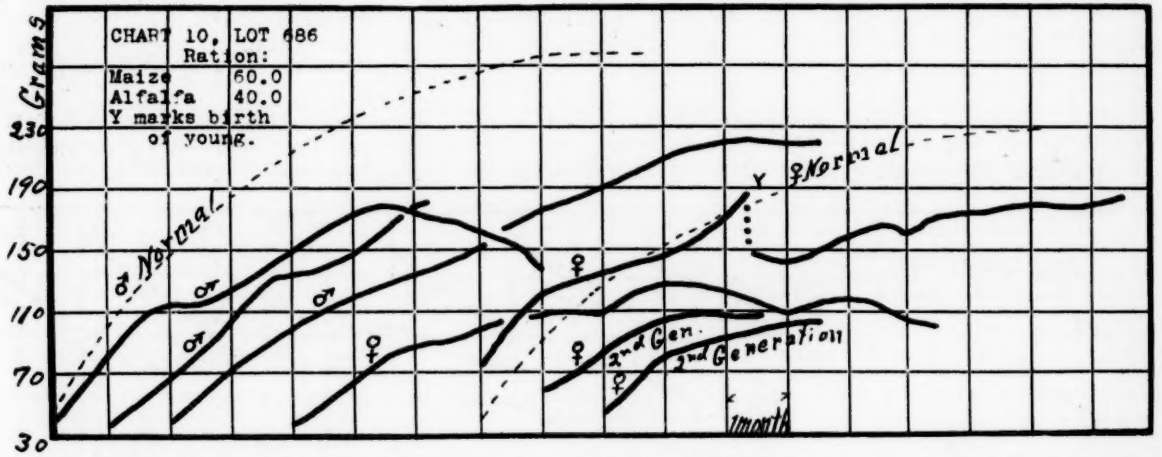

CHART 10. Lot 686 shows distinctly poorer nutrition of animals fed maize 60 and alfalfa 40 than was secured with oats and alfalfa in similar proportions (Chart 6). The growth is, however, much better than we have ever been able to secure with diets restricted to the seeds of plants (Charts 1 to 4). The single litter of young which was secured from the two females of this lot was successfully weaned. The second generation confined to this food mixture is failing to grow. These animals all show distinct signs of old age at 11 months.

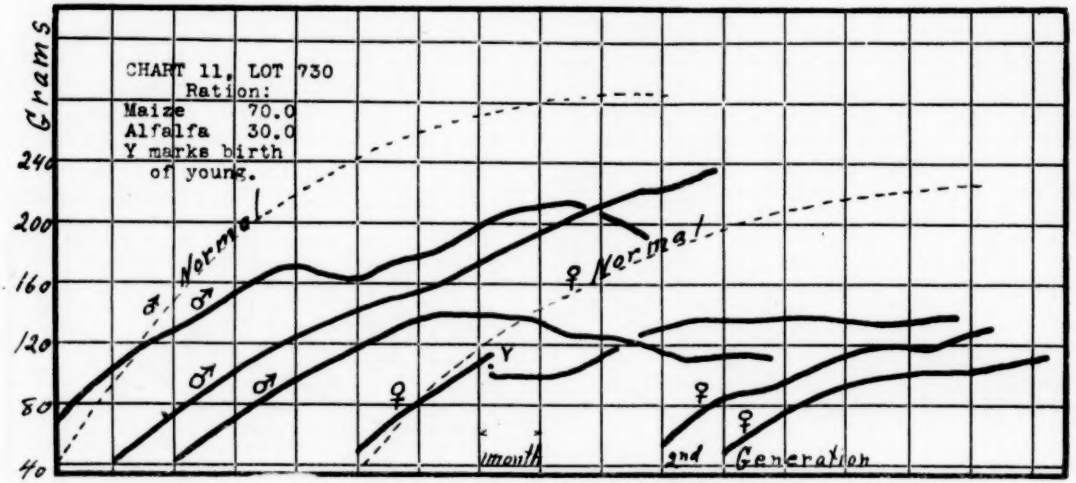

CHART 11. Lot 730. This chart when compared with Chart 10, Lot 686, and with Chart 12, Lot 729, seems to indicate definitely that within certain limits lower planes of alfalfa (10 to 20 per cent) are more successful as supplements to the maize kernel than are higher planes (30 to 40 per cent) such as are most successful with oats (Charts 6 and 7). This is further supported by Chart 10. The reason why the growth and well-being of animals fed maize and alfalfa fall distinctly below the normal must be found by systematic inquiry as described under Chart 9. These records are presented here for the sake of emphasizing the superiority of rations composed of leaf and seed over those made by combining seed with seed (Charts 1 to 4).

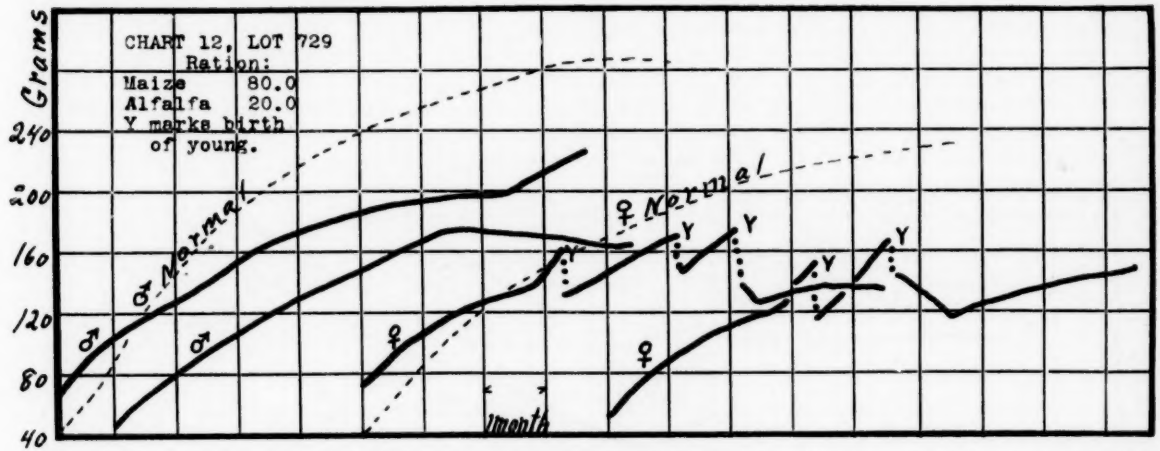

CHART 12. Lot 729. The rats in this group receiving 20 per cent of alfalfa with 80 per cent of maize grew noticeably better than did those receiving higher planes of alfalfa leaf (Charts 10 and 11). Although the mortality of the young was high (eighteen young born and four weaned), the young which survived to an age when they were independent of the mother's milk supply and could eat the mother's ration greatly improved thereafter. Although somewhat undersized, these rats appear to be well nourished. It is hardly probable that growth curves of this character can ever be secured with any combinations of seeds, however complex the mixture, provided salt-free water be given and access to the soil be denied.

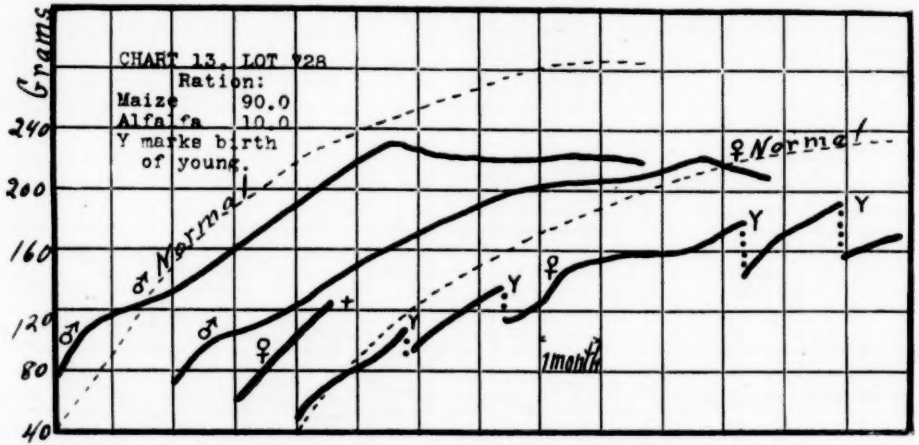

CHART 13. Lot 728. The growth curves of this group are on the whole a little better than those of any other group fed maize and alfalfa. One female died from an unknown cause early in life. The remaining one has produced four litters of young; none of the first three litters were kept alive beyond a few days. Of the fourth litter of eight young, three were reared. These, continued on the ration, weighed 75 gm. at 27 days of age. If fed 100 per cent maize kernel, not the slightest amount of growth would ever be secured. The supplementary relationship of the 10 per cent of alfalfa in this ration is of profound importance to the animal.

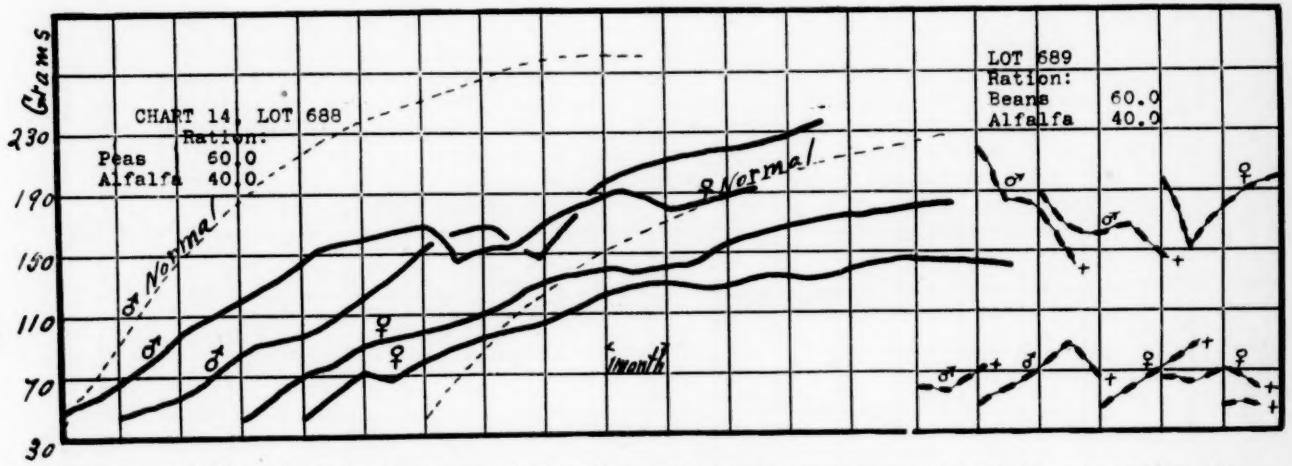

CHART 14. Lot 688. Although rats can grow fairly well on a mixture of peas 60 per cent (soaked in water and heated in an autoclave for 1¼ hours at 15 pounds' pressure) and alfalfa leaf 40, no young have been secured from this group, although it contained two females.

Lot 689. In marked contrast to the growth secured in Lot 688 with 60 per cent of peas and 40 of alfalfa, no growth has been observed in rats fed 60 per cent of beans and 40 per cent of alfalfa. The mortality of animals in this group has been very high in the first few weeks of feeding. A special investigation will be necessary to reveal the reason for this pronounced failure. These results emphasize what has not been hitherto appreciated; *viz.*, that the pea and the bean are very different from the dietary standpoint.

THE PROTEINS OF THE PEANUT, ARACHIS HYPOGÆA.

II. THE DISTRIBUTION OF THE BASIC NITROGEN IN THE GLOBULINS ARACHIN AND CONARACHIN.*

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(From the Protein Investigation Laboratory, Bureau of Chemistry, Department of Agriculture, Washington.)

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The culture of peanuts in the United States has increased rapidly during the last ten years. During the year 1916 a large number of mills which were formerly engaged in pressing cottonseed to obtain oil and press cake were unable to secure a sufficient supply of cottonseed and are now pressing peanuts. Thus, there has been produced a large supply of peanut oil and peanut press cake. The oil, which compares favorably with olive oil for culinary purposes and is also used for making oleomargarine, has found a ready market and was quoted February, 1917, at $1.05 per gallon. The press cake is ground to a meal and is readily finding favor as a stock food. It is quoted at $35 per ton. When this meal is made from whole peanuts it contains about 28 per cent of protein, while meal made from shelled peanuts contains about 45 per cent of protein (N x 6.25).

In a recent paper (1) from this laboratory it was shown that the globulins of the peanut yield a relatively high percentage of basic nitrogen. Arachin, which is the chief protein of the peanut, contains 4.96, and conarachin 6.55 per cent. These values were obtained by the Hausmann method in which no correction is made for the solubility of the phosphotungstates of the bases and are therefore somewhat lower than the figures obtained by the Van Slyke method in which such a correction is applied. These proteins have now been analyzed by the Van Slyke method to ascertain the percentage of the basic amino-acids. The dis-

* Published by permission of the Secretary of Agriculture.

tribution of nitrogen previously reported from analyses by the Hausmann method are given in the following table (2).

N	Arachin.	Conarachin.
Amide	2.03	2.07
Humin	0.22	0.22
Basic	4.96	6.55
Non-basic	11.07	9.40
Total	18.28	18.24

The results of the analyses by Van Slyke's method show that both arachin and conarachin contain arginine, histidine, lysine, and cystine. Both proteins also give a strong qualitative test for tryptophane.

Percentage of Basic Amino-Acids in the Globulins of the Peanut.

	Arachin.	Conarachin.
Arginine	13.51	14.60
Histidine	1.88	1.83
Lysine	4.98	6.04
Cystine	0.85	1.07

The figures for cystine are undoubtedly too low, as they represent only the cystine which escaped destruction during the hydrolysis of the proteins with hydrochloric acid.

The most important fact brought out by these analyses is the high content of lysine in arachin and conarachin. For the sake of comparison the percentages of lysine in the proteins of some common seeds and in muscle from some animals are also given in the following tables.

The figures marked by (§) were obtained by the Van Slyke method and, therefore, probably represent the maximum percentage of lysine obtainable. Those not so marked were obtained by Kossel's absolute method and may be somewhat too low. It is seen, however, that the percentages of lysine in arachin and conarachin of the peanut are relatively high, and, indeed, approach the lysine content of muscle substance of different animals. It will also be noted that no lysine has been found in zein

and that the maximum percentage of lysine obtained from gliadin is only 1.21 per cent.

Osborne and Mendel (3) and other workers have shown that lysine is essential to the growth of animals. Nutrition experiments indicate (4) that the animal organism cannot synthesize lysine which must, therefore, be provided in suitable quantity in the food to insure normal growth. Since the muscle substance of animals contains about 7 per cent of lysine, foods deficient in this essential amino-acid should be supplemented by the

Lysine Content of Some Vegetable Proteins.

Protein.	Source.	Percentage.
Zein.	Maize.	0.00*
Gliadin.	Wheat.	§1.21**
Legumin.	Pea.	4.29†
Phaseolin.	Kidney bean.	4.58‡
Arachin.	Peanut.	§4.98
Conarachin.	"	§6.04

* Osborne, T. B., and Jones, D. B., *Am. J. Physiol.*, 1910, xxvi, 227.

** Osborne, T. B., Van Slyke, D. D., Leavenworth, C. S., and Vinograd, M., *J. Biol. Chem.*, 1915, xxii, 259.

† Osborne, T. B., and Clapp, S. H., *J. Biol. Chem.*, 1907, iii, 219.

‡ Osborne, T. B., *Ergebn. Physiol.*, 1910, x, 116.

*Lysine in Muscle Substance of Different Animals.**

Scallop (*Pecten irradians*)	5.77
Halibut (*Hippoglossus vulgaris*)	7.45
Chicken	7.24
Ox	7.59

* Osborne and Jones, *Am. J. Physiol.*, 1909, xxiv, 438.

addition of other foods which contain a high percentage of lysine. Wheat and corn, both of which contain but little lysine, should therefore prove more efficient diets if supplemented by some food of high lysine content. Peanut meal appears to be well adapted to this purpose. From a nutritive standpoint, it is one of our cheapest foods and seems to possess no objectionable properties. Animals fed on it thrive and increase rapidly in weight (5). It therefore seems probable that corn and wheat could be much better utilized and a considerable saving in the cost of feeding effected by supplementing these cereals with peanut meal.

Peanuts may be used in many ways for human consumption. The Laboratory of Plant Chemistry of the Bureau of Chemistry has found that a mixture of 75 per cent of wheat flour and 25 per cent of peanut flour makes excellent bread. Such a bread is higher in protein content and contains much more lysine than bread made from wheat alone.

Investigations on the proteins of the peanut will be continued.

EXPERIMENTAL.

Analysis of Arachin.—Duplicate samples of 3 gm. of arachin, each equivalent to 2.7210 gm. of ash and moisture-free protein, were hydrolyzed by boiling with 100 cc. of 20 per cent hydrochloric acid for 24 hours. The protein contained 18.28 per cent of nitrogen, equivalent to 0.4974 gm. in each of the samples analyzed. The phosphotungstates of the bases were decomposed by the amyl alcohol-ether method. The following results are corrected for the solubilities of the phosphotungstates of the bases.

Analysis of Arachin. Van Slyke Method.

Total Nitrogen Corrected for Solubility of Bases.

	I.	II.	I.	II.	Average.
	gm.	*gm.*	*per cent*	*per cent*	*per cent*
Amide N	0.0581	0.0594	11.68	11.94	11.81
Humin N adsorbed by lime	0.0028	0.0029	0.56	0.59	0.57
Humin N in amyl alcohol extract	0.0035	0.0007	0.71	0.15	0.43
Cystine N	0.0037	0.0036	0.75	0.73	0.74
Arginine N	0.1182	0.1183	23.76	23.78	23.77
Histidine N	0.0112	0.0166	2.24	3.33	2.78
Lysine N	0.0257	0.0262	5.17	5.28	5.22
Amino N of filtrate	0.2640	0.2662	53.08	53.52	53.30
Non-amino N of filtrate	0.0076	0.0089	1.52	1.79	1.65
Total N regained	0.4948	0.5028	99.47	101.11	100.27

Basic Amino-Acids in Arachin.

	I.	II.	Average.
	per cent	*per cent*	*per cent*
Arginine	13.50	13.51	13.51
Histidine	1.51	2.25	1.88
Lysine	4.93	5.03	4.98

Analysis of Conarachin.—Duplicate samples of 3 gm. of conarachin, each equivalent to 2.6967 gm. of ash and moisture-free protein, were hydrolyzed by boiling with 100 cc. of 20 per cent hydrochloric acid for 24 hours. The protein contained 18.23 per cent of nitrogen, equivalent to 0.4916 gm. in each of the samples analyzed. Ether and amyl alcohol were used to decompose the phosphotungstates of the bases. The following results are corrected for the solubilities of the bases:

Analysis of Conarachin. Van Slyke Method.
Total Nitrogen Corrected for Solubility of Bases.

	I.	II.	I.	II.	Average.
	gm.	*gm.*	*per cent*	*per cent*	*per cent*
Amide N	0.0548	0.0542	11.14	11.02	11.08
Humin N adsorbed by lime	0.0031	0.0033	0.64	0.67	0.65
Humin N in amyl alcohol extract	0.0007	0.0006	0.13	0.12	0.13
Cystine N	0.0046	0.0028	0.93	0.58	0.75
Arginine N	0.1247	0.1288	25.37	26.19	25.78
Histidine N	0.0131	0.0136	2.67	2.78	2.72
Lysine N	0.0320	0.0305	6.51	6.20	6.35
Amino N of filtrate	0.2486	0.2452	50.57	49.88	50.23
Non-amino N of filtrate	0.0089	0.0102	1.81	2.08	1.94
Total N regained	0.4905	0.4892	99.77	99.52	99.63

Basic Amino-Acids in Conarachin.

	I.	II.	Average.
	per cent	*per cent*	*per cent*
Arginine	14.37	14.84	14.60
Histidine	1.79	1.87	1.83
Lysine	6.20	5.89	6.04

SUMMARY.

1. The globulins of the peanut have been analyzed by the Van Slyke method and the results show that they contain the basic amino-acids, arginine, histidine, lysine, and cystine.

2. The relatively high percentage of lysine in the proteins of the peanut indicates that this seed might be used to advantage in supplementing diets deficient in lysine.

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3. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1914, xvii, 325.
4. Hopkins, F. G., *J. Chem. Soc.*, 1916, cix, 629.
5. *Weekly News Letter, U. S. Dept. Agric.*, 1916, iv, 6.

A NOTE ON THE SOLUBILITY OF CHOLESTEROL-DIGITONIDE.

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(Received for publication, March 30, 1917.)

In a review of the colorimetric and gravimetric methods for the determination of cholesterol,[1] it was shown that the colorimetric method of Autenrieth-Funk[2] gave results about 20 per cent higher than those obtained by the Fraser and Gardner[3] modification of the Windaus[4] digitonin method, while a colorimetric procedure proposed by Bloor[5] gave results even as much as 50 per cent higher than the latter. From the experimental evidence it was concluded, however, that the lower gravimetric values were probably more nearly correct. Bloor[6] has recently criticized this conclusion on the basis that the digitonin-cholesterol compound is sufficiently soluble in the ether used for washing to account for the low results.

Owing to the fact that pure cholesterol solutions give practically theoretical results when precipitated by digitonin and washed with ether and hot water, this criticism does not seem well founded. Nevertheless, solubility determinations of the compound in ether and in boiling water have been made and are here presented.

The compound was prepared by precipitating recrystallized cholesterol made from gall-stones, by digitonin, filtering, washing thoroughly with alcohol and ether, and drying. Some of the resulting compound was shaken with ether for 24 hours at room

[1] Mueller, J. H., *J. Biol. Chem.*, 1916, xxv, 549.

[2] Autenrieth, W., and Funk, A., *Münch. med. Woch.*, 1913, lx, 1243.

[3] Fraser, M. T., and Gardner, J. A., *Proc. Roy. Soc., Series B*, 1909, lxxxi, 230.

[4] Windaus, A., *Z. physiol. Chem.*, 1910, lxv, 110.

[5] Bloor, W. R., *J. Biol. Chem.*, 1916, xxiv, 227.

[6] Bloor, W. R., and Knudson, A., *J. Biol. Chem.*, 1916, xxvii, 107.

temperature. After filtering, exactly 100 cc. were evaporated in several portions from a small weighed porcelain dish, the residue dried at 110°, and weighed. As an average of three determinations, 0.0007 gm. was found to have dissolved in 100 cc. of the ether. Similar determinations, using boiling water as solvent, showed a solubility of 0.0006 gm. in 100 cc.

Since approximately 100 cc. each of ether and hot water are used for washing in actual determinations of cholesterol, it is evident from these figures that an error of about 3 per cent might be accounted for in this way on 0.01 gm. of cholesterol (= 0.04 gm. compound), leaving the colorimetric results by Bloor's method still perhaps 45 per cent too high.

UREA AS A SOURCE OF BLOOD AMMONIA.

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(Received for publication, March 6, 1917.)

In the course of a recent study of urea excretion in rabbits it was sometimes found that within ½ to 2 hours after a dose of 5 gm. of urea by stomach tube the animals would have severe general convulsions, usually terminating in opisthotonus and death. In a series of 131 experiments such convulsions occurred in eight instances, while in the remaining animals no symptoms of intoxication were noted. Ammonia poisoning was suspected, since we had seen similar symptoms after the intraperitoneal injection of a urea solution which contained ammonia produced by the action of contaminating bacteria. This suspicion was confirmed when it was found that all rabbits with convulsions in which blood ammonia determinations were made showed a marked increase in ammonia. As much as 11 mg. per 100 cc. were found in one instance, although the urea solution used contained scarcely recognizable traces of ammonia. Attention has been called to the same phenomenon by Bang (1) who found a similar increase of blood ammonia in rabbits after 10 gm. of urea given by mouth, and showed that a blood concentration of about 4 mg. ammonia may be lethal. The same dose of urea given intravenously produced no symptoms, and he therefore concluded that the urea was changed into ammonia by the flora of the large bowel. However, his statement that this conclusion was also confirmed experimentally is not supplemented by any report of the experimental details. Cathcart (2) has recently brought forward evidence which would indicate the possible derivation of ammonia from urea given intravenously. Her figures show a slight increase of liver and muscle ammonia, but are far from convincing when we consider the sources of error of the tissue-ammonia method used.

We have therefore undertaken a brief investigation of the blood ammonia in rabbits following urea given (1) directly into the bowel,

and (2) intravenously, with and without exclusion of the bowel circulation. Serial determinations of the blood ammonia have been made by the method described by one of us (3), and the results are here given as graphs showing the amount of ammonia obtained from the blood at varying intervals following the urea injections.

I. Blood Ammonia Following Direct Injections of Urea into the Digestive Tract.

1. Weight of rabbit 1,200 gm. Ether anesthesia. Midline incision. 3 gm. of urea in 20 per cent solution injeeted directly into the large bowel and appendix. Irregular spasmodic respiration after 45 minutes. Death with respiratory failure at 53 minutes.

2. Weight of rabbit 1,200 gm. Same procedure. Jerky breathing after 30 minutes. Death with respiratory failure at 37 minutes.

3. Weight of rabbit 1,000 gm. Same procedure, with injection of 3.0 gm. of urea into bowel. No symptoms. Bled to death.

4. Weight of rabbit 1,300 gm. 5 gm. of urea (20 per cent) injected directly into the stomach after ligation of the pylorus. No symptoms.

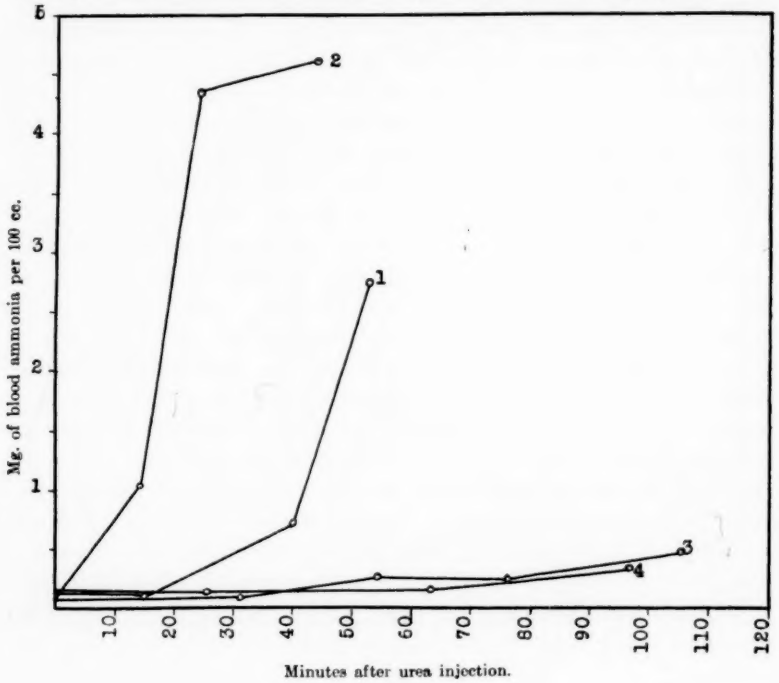

FIG. 1

Results.—Blood ammonia in rabbits shows a rapid rise following direct injection of large doses of urea into the large intestine. No significant rise occurs after urea injection into the stomach with the pylorus ligated.

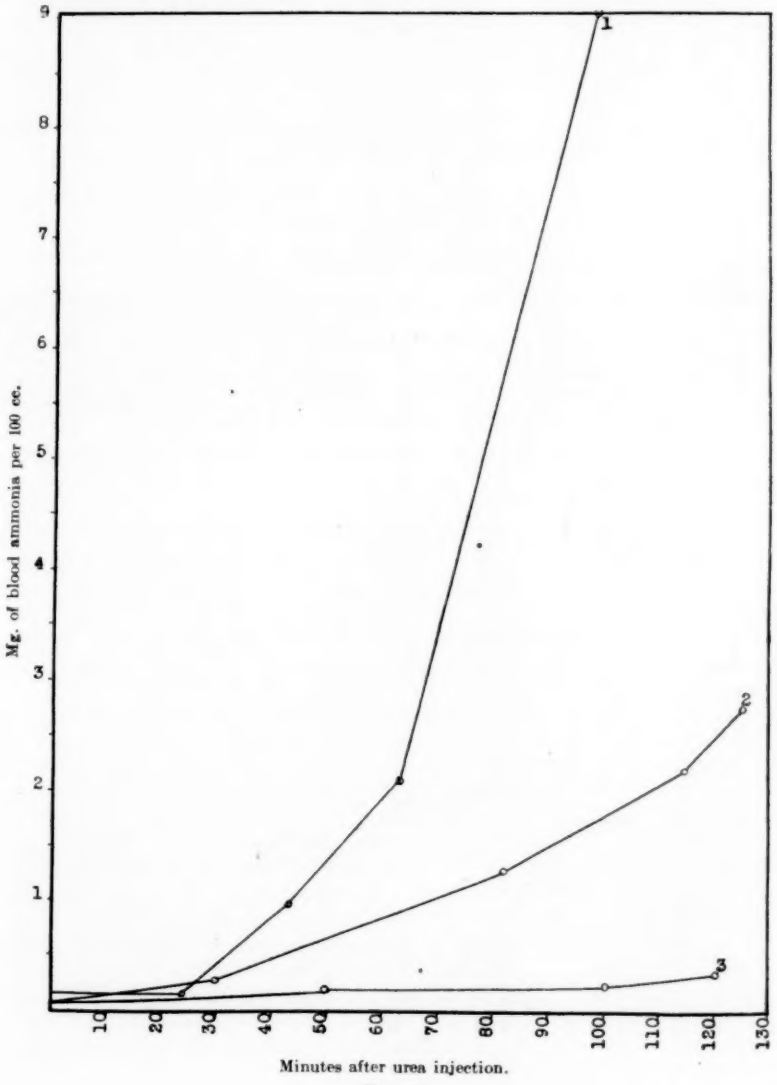

FIG. 2

II. Blood Ammonia Following Intravenous Injections of Urea.

1. Weight of rabbit 1,000 gm. Ether anesthesia. 15 gm. of urea in 25 per cent solution by cannula into the jugular vein during 13 minutes. Death with respiratory failure at 90 minutes.

2. Weight of rabbit 2,050 gm. Ether anesthesia. Celiac axis and superior and inferior mesenteric arteries clamped through lateral abdominal incision. 10 gm. of urea (20 per cent) injected intravenously during 7 minutes. Death from pericardial hemorrhage following removal of a blood sample from the heart.

3. Control. Weight of rabbit 2,250 gm. Vessels clamped as in No. 2. No urea given. Bled to death after 120 minutes.

Results.—The blood ammonia may show marked and rapid rise in rabbits following large doses of urea intravenously. A smaller and delayed rise may occur with the bowel circulation excluded.

DISCUSSION.

The striking increase of blood ammonia following the intravenous urea is, we believe, the clearest evidence that has been brought forward to suggest the possible derivation of ammonia from urea in the body.

The studies of Jacoby (4) and Lang (5) showed a slight urea-splitting activity of tissues *in vitro*, but are of little value, since bacterial action could not be excluded. Eppinger (6) reported that subcutaneous urea will prevent acid intoxication in rabbits when two or three times the lethal dose of hydrochloric acid is given by mouth, and concluded that the acid is neutralized by ammonia obtained from the urea. Pohl and Münzer (7) were unable to confirm Eppinger's experiments, but as noted by Eppinger (8) they gave the urea by mouth, a method which he also found inefficient. Their criticism that the extreme toxicity of ammonia for rabbits would speak against Eppinger's theory seems justified. The marked diuretic action of urea would appear to be a better explanation of its beneficent effect on acid intoxication. Observations on urinary ammonia following urea taken by mouth in man have yielded conflicting results. Janney (9) concluded that no ammonia is formed from the urea taken, although his protocols occasionally show a distinct increase in ammonia output following the urea dose. Lichtwitz (10) and Wolf (11) reported a considerable increase in ammonia output after taking urea, and concluded that the ammonia is formed from the urea. But here, too, the effects of urea diuresis cannot be disregarded. Wakeman and Dakin (12) and Jansen (13) in perfusion experiments found no evidence of a reversibility of the ammonia-urea reaction in the liver. Momose (14), using the oxygen-absorbing

capacity of the blood as a measure of its reaction, concluded that there is an increased alkalinity of the blood following urea given by mouth. The figures before and after urea, however, are apparently within the limits of error of the method.

We would explain the difference between our results with intravenous injection and those of Bang (1), who found no ammonia poisoning, by the fact that we have used larger doses of urea. Furthermore, as he made no determinations of blood ammonia in his intravenous experiments, a considerable sublethal rise may have occurred, a condition which we have found in experiments not here given in detail.

Some of our rabbits have died during or immediately following the urea injections, with general muscular twitching and fine irregular clonic spasms of the extremities, but without increase of blood ammonia. These are apparently due to urea poisoning, and are quite different from the ammonia convulsions, which have only occurred in animals surviving the direct urea intoxication.

It is clear that we have not determined the source of the ammonia. The attempt to exclude the bowel by clamping the arteries is not a satisfactory procedure, and is open to the criticism that the ammonia resulting from the autodigestion of the stomach and small bowel may find its way into the peritoneal cavity and be absorbed there. In fact we found in one such experiment an ammonia content of 26 mg. per 100 cc. in the fluid which accumulated in the peritoneal cavity. The same case showed perforation of the stomach. The delay in the ammonia rise when the bowel is excluded might suggest that the bowel is an important source of the ammonia, and it is of interest to recall that Herter (15) noted in certain animals a transudation of urea into the intestine following intravenous urea. But the rapidity of the rise in ammonia following simple intravenous urea would seem to us to indicate the possibility of some other mechanism. Further work is in progress toward the solution of the problem.

SUMMARY.

1. The blood ammonia of rabbits shows a marked increase following large doses of urea given by mouth, or directly into the bowel, or intravenously. Death from ammonia poisoning frequently follows such injections.

2. A less marked and later rise in blood ammonia may occur following intravenous urea in rabbits in which the bowel circulation is excluded.

3. The possibility that this ammonia may arise elsewhere than in the bowel is pointed out.

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THE INFLUENCE OF THE PROTEIN INTAKE ON THE EXCRETION OF CREATINE IN MAN.

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(Received for publication, March 29, 1917.)

In a previous paper[1] we have shown that in man the excretion of creatine is largely confined to the day, only a small proportion of the total amount of this substance found in the 24 hour urine being eliminated during the night. It was also noted that during the day the output of creatine rose markedly after each meal, thus showing the unmistakable influence of the ingestion of creatine-free food on the excretion of this body. In the present paper we wish to present the results of a series of experiments which we think add further stability to the evidence concerning the influence of protein ingestion on creatine excretion.

In the course of a series of creatine determinations made on the urine of a number of patients suffering from hyperthyroid disease, it was noted incidentally that those patients who were possessed of the insatiable appetite so often seen in the victims of this malady had considerable quantities of creatine in the urine, while persons suffering from an equally severe form of the same disorder, but possessed of little or nearly normal appetite, excreted much smaller quantities of this body. On the working hypothesis that the urinary creatine is dependent on the intake of protein in the food, we have carried out a series of feeding experiments in which patients were fed for varying periods first on the highest protein diet (creatine-free) that they could be induced to take, and then on one containing the minimum amount of protein. The subjects used in the experiments recorded below were kept in bed during the entire period of observation.

[1] Denis, W., *J. Biol. Chem.*, 1917, xxix, 447.

Subject.	Experiment.	Sex.	Age.	Weight.
			yrs.	*kg.*
I	I	F.	33	55.2
II	II	M.	35	58.0
III	III	F.	21	59.0
IV	IV	F.	46	52.3
V	V	F.	23	54.5

All were suffering from hyperthyroid disease of moderate severity and were receiving no drugs during the experimental period.

Observations of body temperature were made at 7 a.m. and 7 p.m. daily, but as no temperatures above normal were recorded these figures have been omitted from the table.

An attempt was made to use practically the same articles of food in all five cases, but personal idiosyncrasies made it necessary to vary to a certain extent the quantity of food given.

During the periods of high protein feeding the following foods were used: bread, a wheat cereal, butter, 40 per cent cream, eggs, cheese, gelatin, sugar, oranges, and apples. During the low protein periods the food consisted of bread, rice, tapioca, butter, 40 per cent cream, potatoes, olive oil, fat bacon, sugar, arrowroot starch, lactose, lettuce, oranges, apples, and bananas.

Creatine and creatinine determinations were made by Folin's micro method,[2] specially purified picric acid being used. The nitrogen determinations were made by the colorimetric method of Folin and Denis.[3]

The figures given in Table indicate gm. of creatine, creatinine, and total nitrogen found in a 24 hour quantity of urine.

As will be seen by an inspection of the figures tabulated below it is apparent that the quantity of protein taken in the food exerts an unmistakable effect on the excretion of creatine in man, a finding in accord with the observations made by McCollum and Steenbock[4] on the pig. Mendel and Rose[5] have shown that the administration of carbohydrate to starving rabbits causes a marked

[2] Folin, O., *J. Biol. Chem.*, 1914, xvii, 472.
[3] Folin and Denis, *J. Biol. Chem.*, 1916, xxvi, 473.
[4] McCollum, E. V., and Steenbock, H., *J. Biol. Chem.*, 1912–13, xiii, 209.
[5] Mendel, L. B., and Rose, W. C., *J. Biol. Chem.*, 1911–12, x, 213, 255.

diminution in the excretion of creatine. Arguing from the results obtained by these investigators, it might be claimed that the high creatine figures found during the periods of forced protein feeding are due to a lack of carbohydrate in the diet. While the fact that during the periods of low protein feeding we not

TABLE I.

Day.	Experiment I.			Experiment II.			Experiment III		
	Total nitrogen.	Prepared creatinine.	Creatine.	Total nitrogen.	Prepared creatinine.	Creatine.	Total nitrogen.	Prepared creatinine.	Creatine.
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	9.1	0.66	0.26	10.2	1.22	0.46	11.9	0.84	0.64
2	11.2	0.74	0.48	8.7	1.25	0.22	12.2	0.78	0.65
3	9.2	0.69	0.54	6.6	1.24	0.19	11.1	0.82	0.53
4	12.7	0.71	0.61	13.2	1.20	0.34	10.4	0.81	0.48
5	7.0	0.68	0.56	20.7	1.33	0.75	8.8	0.80	0.48
6	6.5	0.69	0.29	21.8	1.29	0.79	8.5	0.83	0.31
7	5.6	0.68	0.11				6.6	0.78	0.27
8	4.7	0.68	0.08				6.3	0.78	0.32

Day.	Experiment IV.			Experiment V.		
	Total nitrogen.	Prepared creatinine.	Creatine.	Total nitrogen.	Prepared creatinine.	Creatine.
	gm.	gm.	gm.	gm.	gm.	gm.
1		0.62	0.03	14.44	0.68	0.39
2	8.5	0.62	0.03	11.07	0.71	0.29
3	12.2	0.58	0.16	11.0	0.70	0.32
4	13.7	0.60	0.24	10.8	0.70	0.32
5	11.4	0.62	0.25	7.0	0.68	0.16
6	12.1	0.75	0.25	5.5	0.74	0.00
7	8.08	0.74	0.19			
8	4.16	0.60	0.00			

only decreased the intake of protein but also increased the consumption of carbohydrates cannot be denied, we feel the above objection to be invalid, as, on looking over the diet lists, it is apparent that at least 300 gm. of carbohydrate were consumed daily by all the subjects during the high protein periods, an amount certainly sufficient to preclude the idea of "carbohydrate starvation."

The conditions under which creatine excretion has been noted in man, *viz.*, in starvation, in childhood, in pregnancy, in fevers, and in hyperthyroid disease, are all conditions accompanied by a high level of protein metabolism. The observations of Krause,[6] that small quantities of creatine are frequently found in the urine of women, may also possibly be attributed to the relatively high protein intake of women when considered from the standpoint of their muscular development.

It was pointed out some 10 years ago by Folin that the results obtained in feeding experiments with creatine depend entirely on the previous diet of the subject. If the food had been low in protein, a marked retention of creatine was observed; if high, most of the ingested creatine was found in the urine.

As a working hypothesis it may be suggested, therefore, that on the ingestion of protein some fraction of this is transformed into creatine, transported to the muscles, and there absorbed.[7] If so much creatine is manufactured that the muscles become supersaturated, creatine is excreted by way of the kidney. What would constitute supersaturation would depend not only on the total mass of muscle, but on the capacity for creatine absorption possessed by these muscles. Thus it will be noted in the experimental results presented above that the first day of high protein feeding seldom brings about much of an increase in the creatine excretion; it is only after a day or two of this diet that a marked increase in excretion is observed.

It must also be borne in mind that the muscles of different individuals on the same diet may and probably do have different saturation points. It has been pointed out by Folin and Denis[8] that if, as they suggest, the creatine found in the urine of children is due to an excessively high level of protein consumption, then it should be possible to reproduce in adults by forced feeding with protein the condition with reference to creatine found in children. It is exceedingly improbable, however, that any creatine excretion would result were the diet given to these hyperthyroid patients eaten by a normal woman. It would therefore seem possible that in this condition and in some other pathological condi-

[6] Krause, R. A., *Quart. J. Exp. Physiol.*, 1911, iv, 293.
[7] Folin and Denis, *J. Biol. Chem.*, 1914, xvii, 493.
[8] Folin and Denis, *J. Biol. Chem.*, 1912, xi, 253.

tions, as well as in childhood, the saturation point of the muscle for creatine is low, a hypothesis supported by the low values for muscle creatine found in children and in persons suffering from exophthalmic goiter.[9]

SUMMARY.

Results are presented on five cases of Graves' disease in which it is shown that the amount of creatine excreted by these individuals is dependent on the intake of protein, being increased by high protein feeding and decreased or even eliminated by low protein feeding.

[9] Denis, *J. Biol. Chem.*, 1916, xxvi, 379.

INHIBITION OF DIGESTION OF PROTEINS BY ADSORBED TIN.

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INTRODUCTION.

It is known that tin is present in foods which have been packed in tin cans, and, since it is taken into the body, the question of the toxicity of the tin is important. Determinations of the toxicity of soluble tin salts have been made by a number of investigators.[1] We have shown in a previous paper that, in some of these foods, the tin removed from the lining of the can remains in true solution; that is, in a dialyzable form.[2] In the case of such foods, determinations of physiological action, as usually made, would apply. Many fruits and vegetables, however, while containing little acid, still dissolve large quantities of tin and in these the greater percentage of the tin has been shown to be present in an insoluble form, neither dialyzable nor filterable. This was especially true in foods whose protein content was high. We found, too, that this tin was very firmly bound, and, judging from the stability of this tin-protein complex, we suggested the possibility that it might pass through the body without being broken up by digestion. In order to secure evidence on these points, artificial gastric and tryptic digestions of proteins have been carried out in the presence and absence of adsorbed tin.

EXPERIMENTAL.

Several methods were available for the study of proteolytic action, the applicability of each depending on the nature of the in-

[1] White, T. P., *Arch. exp. Path. u. Pharm.*, 1880–81, xiii, 53. Ungar, E., and Bodländer, G., *Z. Hyg.*, 1887, ii, 241. Salant, W., Rieger, J. B., and Treuthardt, E. L. P., *J. Biol. Chem.*, 1914, xvii, 265.

[2] Goss, B. C., *J. Ind. and Eng. Chem.*, 1917, ix, 144.

vestigation. Since we were only interested in comparing the rate and extent of proteolysis of proteins containing adsorbed tin with that of the normal protein, a method which gave relative results and could be rapidly carried out was desirable. The biuret reaction, as described by Sherman and Neun in a recent review of methods,[3] fulfilled these conditions, the gradual change in color from violet to rose-red indicating the formation of peptones. The methods which depend on a change in hydrogen ion concentration, such as the formol titration, were not applicable here because of the fact that adsorption phenomena occurred in the case of the tin-protein complex, producing a change in acidity when no enzyme was present. For example, a series of albumins, containing varying amounts of adsorbed tin, was placed in contact with a 0.5 per cent Na_2CO_3 solution and it was found that as the percentage of tin increased, the decrease in acidity became more marked, although no tin went into solution.

TABLE I.

Decrease in Acidity of Solutions in Contact with Tin-Protein Adsorption Complex.

8 gm. of albumin in 150 cc. of 0.5 per cent Na_2CO_3. 25 cc. of this solution were found equivalent to the following volumes of 0.1 N HCl.

Time. *hrs.*	0	½	1	Decrease in HCl.
Mixture.	0.1 N HCl required.			
	cc.	cc.	cc.	cc.
Albumin without tin	9.55	8.75	8.65	0.9
" with 1.16 per cent tin	9.55	8.15	7.4	2.15
" " 32.8 " " "	9.6	6.65	4.3	5.3

This phenomenon will receive further attention.

The biuret reaction, taken together with the visible differences in the amounts of coagulated proteins left undissolved at any period of time, and supplemented by tin determinations on the digestion liquor and residue, was found to give interesting results from the point of view indicated above.

The proteins studied were egg albumin, edestin, casein, and the 10 per cent NaCl extract of globulins (and albumins) from pea-

[3] Sherman, H. C., and Neun, D. E., *J. Am. Chem. Soc.*, 1916, xxxviii, 2199.

beans, each in the solid form. The egg albumin was prepared by boiling eggs for 10 minutes, removing the albumin, and pressing it through a 20 mesh gauze, thus obtaining samples of uniform surface and water content. 7 gm. samples of this albumin were then weighed out and placed in a series of flasks, 100 cc. of a solution of stannic ammonium chloride in 1 per cent HCl being added to each, the concentrations ranging from 10 to 0.01 per cent. After remaining in contact with the albumin for 12 hours, during which time the mixture was frequently stirred, a 10 cc. portion of the solution was withdrawn from each concentration and analyzed for tin; then the tin solutions were drained off and the albumin

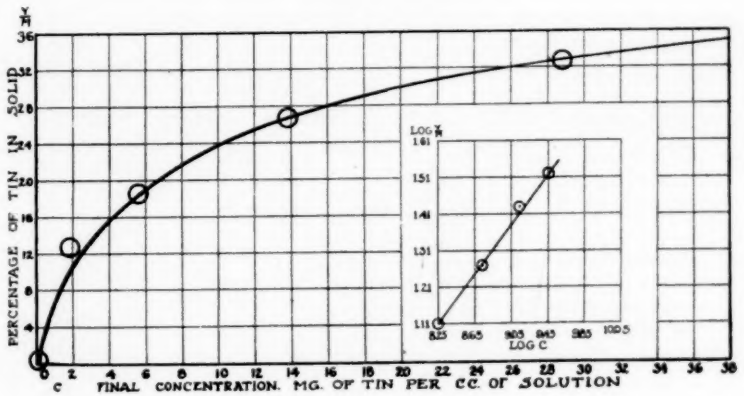

FIG. 1. Adsorption of tin by coagulated egg albumin.

was washed repeatedly with distilled water, finally being left over night under distilled water, this water giving no test for tin. A part of the solid was dried at 110°C. and the percentage of tin determined, after destruction of the organic matter, by precipitating as sulfide from acid solution, the sulfide being roasted and weighed as SnO_2. From these results the adsorption curve was plotted.

The heat-coagulated globulins from beans, crystallized edestin, and the acid-precipitated casein from milk were all treated in a similar way and were all found to adsorb tin in varying degrees.

Equal volumes (100 cc.) of a solution of 0.5 per cent pepsin in 0.05 N HCl were added to the protein and tin-protein samples

and the flasks placed in a thermostat at 38°C. At intervals the filtrate was tested by the biuret reaction and the appearance of the solid noted. After the experiment was finished, the percentage of tin in the solid residue was determined and the liquor tested for filterable and dialyzable tin.

In the same way another series was treated with a solution of trypsin, 0.5 per cent, in 0.1 N Na_2CO_3.

Some typical results follow.

TABLE II.

Peptic Digestion of Egg Albumin as Indicated by the Biuret Reaction.

Time.	Percentage of tin in coagulated albumin.						
	0 Boiled.	0	1.16	12.8	18.5	26.8	32.8
hrs.							
1	Violet.	Red.	Red-violet.	Violet.	Violet.	Violet.	Violet.
2	"	"	Violet-red.	Red-violet.	"	"	"
6	"	"	Red.	Violet-red.	Red-violet.	Red-violet.	"
18	"	"	"	Red.	Violet-red.	Violet-red.	Red-violet.
					Residue, 50.5 per cent tin. Dialyzable tin, none. Filterable tin, traces.		Residue, 48.2 per cent tin. Dialyzable tin, none. Filterable tin, traces.

The albumin containing no tin was almost completely dissolved after 2 hours, while the albumin with 26 and 32 per cent tin showed little change in volume at the end of 18 hours. The volume of the residue after 18 hours was proportional to the percentage of tin in the albumin at the beginning of the experiment.

TABLE III.

Coagulated Egg Albumin.
Tryptic Digestion as Indicated by the Biuret Reaction.

Time.	Percentage of tin in coagulated albumin.						
	0 Boiled.	0	1.16	12.88	18.53	26.85	32.8
hrs.							
1	Violet.	Red-violet.	Reddish violet.	Violet.	Violet.	Violet.	Violet.
2	"	Violet-red.	Red-violet.	Reddish violet.	"	"	"
4	"	"	"	Red-violet.	Red-violet.	Red-violet.	"
6	"	"	Violet-red.	"	"	"	Reddish violet.
18	"	Red.	"	Violet-red.	"	"	Red-violet. Residue 62.7 per cent tin. Dialysate, no tin. Filtrate, no tin.

In tin 0 per cent there was almost complete solution after 4 hours, while in tin 32 per cent there only was a slight apparent volume change after 18 hours.

TABLE IV.

Casein, Peptic Digestion.

Time.	Percentage of tin in casein.			
	0	1.02	8.5	21.4
hrs.				
3	Violet-red.	Violet.	Violet.	Violet.
5	Red.	Red-violet.	"	"
9	"	Violet-red.	Violet-red.	Red-violet.
18	"	Red.	Red.	Violet-red.

TABLE V.

Edestin, Tryptic Digestion.

Time.	Tin 0, boiled.	Tin 0.	Tin-protein from 5 per cent solution.
hrs.			
1	Violet.	Violet-red.	Violet.
2	"	Red.	"
3	"	"	Red-violet.
18	"	"	Violet-red.

After 18 hours the normal edestin was almost completely dissolved while there was no apparent change in the tin-edestin.

The results given by the biuret reaction in the case of the globulins were not decisive, but, while the normal globulin was almost completely dissolved after 6 hours, the tin globulin showed little or no change after 18 hours.

TABLE VI.

Peptic Digestion, Egg Albumin Containing 37 per Cent Tin.

Time.	Tin 0, boiled.	Tin 0.	Tin 37 per cent.
hrs.			
2	Violet.	Red-violet.	Violet.
8	"	Red.	"
24	"	"	"
48	"	"	Red-violet.
100	"	"	Red.

After 100 hours there was almost no change in the volume of the solid albumin in the digestion mixture in the flasks containing the tin-protein complex. A duplicate which was examined at the end of 48 hours was found to have no dialyzable tin, 3 mg. of filterable tin, and 1,175 mg. of tin in the washed solid residue.

CONCLUSIONS.

We have found that tin is readily adsorbed from solutions by coagulated proteins in amounts varying with the concentration according to the adsorption law of Freundlich; that this adsorption takes place rapidly at first and then continues to increase slowly for several days, presumably because of the time required for diffusion into the solid. The adsorption complex is extremely stable and does not lose tin to a dilute acid or alkaline aqueous phase containing no tin although the percentage of tin in the solid

may be as high as 50 per cent. The presence of this tin, even in small amounts, interfered markedly with the digestion of the protein by either peptic or tryptic digestion, as was indicated by the change in color of the biuret reaction and by the visible retardation of the solution of coagulated proteins. At the end of a 6 hour digestion, under exactly the same conditions, it was possible to arrange a series of albumins in the order of the increasing percentages of tin, by observing the extent to which solution had taken place. It was observed, however, that when the time was increased a certain amount of solution or digestion did occur even when the protein contained large amounts of tin, and that in the case of the proteins of low tin content, comparable with the condition present in a can of food, only a small residue was left undissolved. This residue contained practically all the tin. Also, if a sample of albumin was left but a short time in contact with a strong tin solution before being subjected to digestion, the residue presented a more or less shell-like structure. It appears from these observations that it is only a part of the protein, directly joined to the tin, whose digestion is hindered. The effect of this retardation, on the food value of a protein containing tin, can only be a matter of conjecture but it is probably small since the total tin in foods rarely exceeds 0.03 per cent.

The other aspect of the problem is more important; namely, that of the possible toxicity of this tin-protein complex. Food rarely remains in contact with the digestive ferments in the body for a longer period than 4 to 6 hours. We have found that even after 48 hours, in the case of the artificial peptic digestions of the tin-albumin containing 32 per cent tin, there was no tin in a dialyzable form, and only a very small amount in a filterable form, this probably being present as a colloidal suspension of particles of the tin-protein complex, split away from the solid by solution of the connecting albumin. In the tryptic digestions no dialyzable tin was found in the liquor and only traces of filterable tin. Moreover, during digestion the percentage of tin in the solid rapidly increased, indicating that the protein which was not directly bound to the tin was being dissolved away. A sample of albumin which originally contained 18 per cent of tin was found after 24 hours' digestion to contain 50 per cent; one with 32 per cent increased to 62 per cent. The activity of the

enzyme was shown to be unaffected by the tin in the proteins by removing some of the liquor from the undigested tin-protein after 100 hours and finding that fibrin was completely and quickly dissolved by the liquor.

We have found that the insoluble complex, formed when coagulated proteins are brought into contact with solutions containing tin, was not broken up to any extent by our artificial gastric and tryptic digestions. The two enzymes, peptin in the stomach and trypsin in the duodenum, are the ones which bring about proteolytic action in the body. Erepsin does not attack proteins themselves, but hydrolyzes the peptones and polypeptides which are formed by the partial splitting of the proteins. While it is impossible to predict what the action of bacteria in the stomach and intestine would be, it is to be questioned whether the tin-protein complex is broken up in the actual digestive processes in the body and whether the tin which is combined in this way can have any toxic action.

SEDOHEPTOSE, A NEW SUGAR FROM SEDUM SPECTABILE. I.*

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(Received for publication, March 31, 1917.)

Sedum spectabile belongs to the natural order of *Crassulaceæ*, the members of which are widely distributed throughout the warm and temperate zones. It is a cultivated herbaceous perennial plant, very hardy, is extremely easily propagated by cuttings or division of the roots, and is commonly used in many countries as an ornamental plant. The order has been but little studied chemically.

Indications of the Presence of a Sugar Having Peculiar Properties.

It was first noticed that an aqueous extract of the crushed fresh leaves and stalks of the plant, prepared during the growing season, from May to September, contained considerable amounts of a free reducing sugar, which was not fermentable with yeast and gave a strong test with orcin and hydrochloric acid, indicating the presence of a pentose or heptose sugar.

It was next sought to learn whether the sugar is an aldose or a ketose. To determine whether one or both of these types are present in a solution, two methods may be applied. In general, ketoses are destroyed by boiling with hydrochloric acid, leaving any aldoses which may be present affected only slightly or not at all. By determining the copper-reducing power of the sugar solution before and after such treatment, a clue to the amounts of aldehydic and ketonic sugars may be obtained.

A second method, which may serve as a control to the first, consists in allowing bromine to act on the sugar solution. This

*Our thanks are expressed to Mr. T. Yoshikawa for valuable assistance rendered during part of this work.

reagent oxidizes aldoses to non-reducing monobasic acids while ketoses remain unaffected.

The plant extract was therefore subjected to both these treatments. In each case a loss in power to reduce Fehling's solution was observed and the amount of Cu_2O formed from a given volume of solution after treatment with either acid or bromine was about 20 per cent of the amount from the same quantity of the original solution.

These results are unintelligible on the assumption that a mixture of aldoses and ketoses of the usual type is present in the plant extract, because the result of the action of hydrochloric acid would indicate that the mixture consisted of 20 per cent aldose and 80 per cent ketose, while the change caused by bromine would lead one to the opposite conclusion.

In both instances similar changes in the optical rotation of the two solutions were observed. From an original reading of about 0°, both treatments caused the solutions to become strongly levorotatory. These puzzling relations made it seem probable that a sugar was present in the *Sedum* plant which exhibited abnormal behavior and it was decided to submit the substance to a thorough investigation. Consequently, a large amount of the plant was treated according to the following method.

Preparation of a Sugar Sirup from the Plant.

This preparation may be best illustrated by the following experiment on a small scale, in which 1,650 gm. of the fresh leaves and stalks of *Sedum spectabile* were used. The material was passed through a chopping machine, an equal volume of water added, and the liquid allowed to drain over night through cloth. Most of the remaining liquid was extracted from the residue by means of a powerful press and the operation was repeated once after moistening the material with about 500 cc. of water. The combined extracts were boiled with a little decolorizing carbon, filtered on folded filters, and then concentrated under reduced pressure to a thin sirup. This was diluted with 95 per cent alcohol until a permanent precipitate was produced, and then poured into four or five times its volume of alcohol of the same strength. The voluminous light colored material which separated was fil-

tered off with suction and washed by thoroughly mixing with alcohol and again filtering. The combined filtrates were evaporated under diminished pressure to a thick sirup. Portions of about 250 gm. of this material were diluted with ten parts of water and in the solution 5 to 6 gm. of tannic acid were dissolved, and basic lead acetate solution was then added as long as a precipitate was produced.[1] This was removed by filtration with suction and the filtrate, after removal of the lead with hydrogen sulfide, was concentrated under diminished pressure to a thick sirup which was used directly in some of the following experiments and is referred to as "crude sirup." It may be purified to a certain extent in the following manner. 70 gm. were dissolved in 700 cc. of water, the solution was cooled by cracked ice, and then made slightly alkaline by addition of a warm solution of barium hydroxide. The solution was then neutralized with carbon dioxide and concentrated to a thin sirup (about 140 cc.). As much 95 per cent alcohol was added as was possible without producing a permanent precipitate and the solution was poured into 2 liters of absolute alcohol which was being vigorously stirred. The separated white material, which settled readily, was filtered off with suction and washed with alcohol. The filtrate on evaporation under diminished pressure yielded a clear light yellow sirup which will be referred to as "purified sirup."

In preparations on a large scale, much greater quantities than the above mentioned amount of the plant were worked up and several kilos of "crude sirup" prepared.[2] Calculated as glucose from the reducing power of the extracts, there was about 1.5 per cent free sugar present in the fresh plant.

Action of Hydrochloric Acid and of Bromine on the Sirups.

A solution containing about 10 per cent of the purified sirup showed only a slight dextrorotation, but when it was slightly acidified with hydrochloric acid and allowed to stand for several

[1] Schorger, A. W., and Smith, D. F., *J. Ind. and Eng. Chem.*, 1916, viii, 495.

[2] Acknowledgments are made to Mr. Xavier Schmitt, Horticultural Hall, Fairmount Park, Philadelphia, to Thomas Meehan and Sons, Inc., Germantown, Pa., and to Wm. H. Moon and Company, Morrisville, Pa., for supplying large quantities of *Sedum spectabile*.

hours or warmed for a short time on the steam bath, its rotation was reversed and greatly increased in magnitude. At the same time its reducing power towards Fehling's solution decreased regularly with the increase in levorotation. These relations in a 10 per cent solution may be illustrated by the following table.

HCl content.	Time of heating.	α in 2 dm. tube.	Cu_2O from 1.66 cc.
per cent	*min.*		*gm.*
0	0	+ 0.5°	0.2976
1	7	− 6.1°	0.2003
1	14	−12.5°	0.1241
1	60	−19.2°	0.0603
1	90	−19.8°	0.0630

It is apparent from the changes in properties of the sugar solution that some reaction has occurred. This is probably not the destruction of some ketose which might be present, because sugars of this type are not much affected by such mild treatment, and, besides, their destruction by hydrochloric acid is always indicated by coloring, whereas solutions of the sugar from the *Sedum* plant remained perfectly clear and did not darken during the process.

The possibility that the change was the result of hydrolysis of a complex carbohydrate must also be excluded because, obviously, such a reaction would be accompanied by an increase in reducing power instead of a decrease as observed.

The reaction which occurs apparently reaches a definite endpoint after 1 hour, since prolonged heating produces no further change and the ratio of the reducing power of the material in the final solution to that in the original remains about as 20: 100.

If a solution of the sugar thus transformed is treated with an excess of bromine, no further changes in reducing power or rotation are produced. The action of this reagent was then tested on a solution of the untransformed sugar sirup.

Bromine causes a solution of the sirup to undergo a change that appears to be substantially the same as that produced by dilute hydrochloric acid. Such a solution, having an initial reducing power of 0.1360 gm. of Cu_2O per 1.66 cc. and an initial rotation of +0.4° in a 2 dm. tube, was allowed to stand with an excess of

bromine. After 3 days the excess of the reagent was removed with a stream of air, and the solution then showed a rotation in a 2 dm. tube of -10° and a reducing power of 0.0416 gm. Cu_2O per 1.66 cc. In this case, as well as in that of the transformed sugar solution, much of the bromine had been used up and appeared partly as free hydrobromic acid in the solution. At the same time, no organic acids were produced, because after removal of the halogen with silver carbonate and the slight excess of silver with hydrogen sulfide the solution proved to be nearly neutral and no evidence of the presence of a lactone could be obtained. Evidently the action of bromine is one of substitution, probably in some organic substance present in the solution other than the sugar, and liberation of hydrogen bromide, which then acts upon the unchanged sugar present in the same manner as hydrochloric acid.

It was concluded from these experiments that there is present free in the *Sedum* plant a reducing sugar which undergoes in dilute hydrochloric acid solution a change by which its optical rotation is completely altered and its reducing power largely decreased. It was thus apparent that the sugar exhibits properties which have not hitherto been observed in this group, and it was next sought to obtain crystalline derivatives of it, especially those with the phenylhydrazines.

Behavior of the Sugar towards the Phenylhydrazines.

Phenylosazone.—All attempts to prepare a hydrazone under varied conditions resulted in the formation of the osazone. This derivative was prepared with ease by the usual method from about a 2 per cent solution of the sirup. It also formed in solutions at room temperature when they were allowed to stand for a few hours with phenylhydrazine acetate. The yield was better than that usually obtained from other sugars, and even from sugar solutions that had been heated with dilute hydrochloric acid the same osazone was obtained and the amount about corresponded to the final reducing power of the solution; that is, it was about 20 per cent of the amount that could be obtained from the solution before transformation by acid. The compound was recrystallized by dissolving it in hot methyl alcohol, filtering, and concen-

trating the solution to a small volume. The substance melted with decomposition at 197° (uncorrected).

The analysis corresponds to the phenylosazone of a heptose.

0.1407 gm. substance gave 0.3031 gm. CO_2 and 0.0797 gm. H_2O.
0.1366 " " " 17.95 cc. N_2 at 28° and 764 mm.
0.1269 " " " 17.0 " " " 29° " 765 "

	Calculated for $C_{19}H_{22}O_4N_4$:	Found:
C	58.76	58.75
H	6.19	6.29
N	14.43	14.66 14.65

p-Bromophenylosazone.—All efforts to prepare a *p*-bromophenylhydrazone in aqueous alcoholic and glacial acetic acid solution yielded the corresponding osazone.

100 cc. of a 2 per cent aqueous solution of the sugar sirup were heated for 3 hours on the steam bath after addition of 5 gm. of *p*-bromophenylhydrazine in 4 cc. of acetic acid. The osazone crystallized from the hot solution in bright yellow needles and was recrystallized from methyl alcohol in the same manner as was the unsubstituted osazone. It melted with decomposition at 227–228° (uncorrected). The analysis corresponds to the bromophenylosazone of a heptose.

0.2577 gm. substance gave 0.1766 gm. AgBr.
0.2466 " " " 0.1692 " "
0.1610 " " " 15.6 cc. N_2 at 30° and 763 mm.

	Calculated for $C_{19}H_{22}N_4Br_2O_4$:	Found:
Br	29.30	29.16 29.18
N	10.26	10.50

No crystalline methyl-, benzyl-, or *p*-nitro-phenyl hydrazone could be prepared under any conditions tried.

The Osone of the Sugar of the Sedum Plant.—The ease with which the new sugar combined with phenylhydrazine indicated the possibility of its being of the nature of an osone rather than of a true sugar. In order to compare this derivative with the sirup obtained from the plant extract the phenylosazone was decomposed according to Fischer's method for preparing glucose

osone.[3] The only changes which were introduced in his directions consisted in omitting to warm the solution of the osazone in concentrated hydrochloric acid, and, in precipitating the lead compound of the osone, a small amount of lead acetate solution was added before precipitation with barium hydroxide. The product obtained from the lead compound was a yellow sirup which did not crystallize. It reduced Fehling's solution strongly and rotated slightly to the left, but did not exhibit changes in hydrochloric acid similar to those described in the case of the reducing substance from which the osazone was derived. On treatment with phenylhydrazine acetate it yielded the original osazone.

Compound of the Osone with o-Phenylenediamine. This derivative was prepared by adding a hot aqueous solution of 2 gm. of *o*-phenylenediamine to about 4 gm. of osone sirup in 60 cc. of water. The slightly colored solution was warmed for 45 minutes on the steam bath and deposited long white needles on cooling. The yield was about 2 gm. The substance was recrystallized from hot water and dried over night in a desiccator. It melted with decomposition at 163–165° (uncorrected) after it had begun to shrink at about 160°. The compound appears to contain one-half molecule of water of crystallization.

0.1519 gm. air-dried substance, when heated to constant weight at 100° *in vacuo* over P_2O_5, gave 0.0042 gm. H_2O.

0.1340 gm. air-dried substance gave 0.0033 gm. H_2O.

	Calculated for $C_{18}H_{18}N_2O_5 + \frac{1}{2} H_2O$:	Found:
H_2O	3.12	2.75 2.46

0.1477 gm. dry substance gave 0.3023 gm. CO_2 and 0.0780 gm. H_2O.
0.1307 " " " " 0.2680 " " " 0.0673 " "
0.1382 " " " " 12.3 cc. N_2 at 24° and 765 mm.

	Calculated for $C_{18}H_{18}N_2O_5$:	Found:	
C	55.71	55.81	55.87
H	5.70	5.86	5.72
N	10.00	10.00	

The analysis thus corresponds to the phenylenediamine derivative of the osone of a heptose. It was not possible to obtain

[3] Fischer, E., *Ber. chem. Ges.*, 1889, xxii, 87.

this derivative from the original sugar sirup and therefore it was concluded that the reducing substance could not be a compound of the osone type.

Since the analyses of the three crystalline derivatives that have been described show conclusively that the sugar of *Sedum spectabile* contains seven carbon atoms, it will be named, with reference to its origin, *sedoheptose*.

Reduction of the Sugar Sirup with Sodium Amalgam.

In order to obtain less complicated crystalline derivatives of the new sugar and also with the object of determining if possible whether it is of aldehydic or ketonic nature, it was next sought to prepare the corresponding heptitol or heptitols by reduction with sodium amalgam. Since aldoses give on reduction only one alcohol while ketoses yield two isomeric ones, the two types of sugars may be distinguished by their behavior on this treatment.

From the sugar sirup two seven carbon alcohols were obtained and the inference would therefore be that it contained a ketose. However, as the material used for reduction was a sirup of uncertain purity there still remained the possibility that it contained two sugars. On the other hand, only one phenylosazone could be obtained from the sirup, and therefore if more than one sugar is present the mixture would seem to be limited to two epimeric aldoheptoses and the corresponding ketose. This point will be discussed later on, when it will be shown that the reducing substance is almost certainly a single ketose sugar.

α-Sedoheptitol.—28 gm. of purified sirup were dissolved in 150 cc. of water, and the solution was cooled by surrounding it with ice and was reduced with sodium amalgam. A small amount of litmus was added as an indicator and the solution was neutralized from time to time with sulfuric acid. The reduction, which was always carried out on the alkaline side, required several days action of the amalgam for completion. It was found convenient to make the reduction in an open vessel of heavy glass, keeping the solution and amalgam agitated with a mechanical stirrer. When the liquid showed no reduction of Fehling's solution, or only a trace, it was made slightly acid to Congo paper, separated from the mercury, and concentrated under reduced pres-

sure until a large part of the salts had separated. It was then mixed with a large volume of hot 95 per cent alcohol, a little decolorizing carbon was added, and the separated salts were removed by filtration with suction. The clear solution yielded on evaporation a nearly colorless sirup which crystallized partially after several weeks standing. If crystals for seeding are available, this time can be shortened to a few days. The crystals were stirred with a little 90 per cent alcohol, filtered off, and washed with a little of the same solvent. The yield was 7.5 gm. The substance was recrystallized by dissolving in a small amount (one to two parts) of hot water, filtering, and adding several volumes of hot absolute alcohol. It melted sharply without decomposition at 151–152° (uncorrected). It was again recrystallized in the same manner as described and showed no change in melting point. The analysis agrees with the composition of a heptitol.

0.1340 gm. substance gave 0.1941 gm. CO_2 and 0.0900 gm. H_2O.

	Calculated for $C_7H_{16}O_7$:	Found:
C	39.63	39.56
H	7.54	7.46

0.4857 gm. substance in 5 cc. H_2O in a 1 dm. tube showed a dextrorotation of +0.21°; hence $[\alpha]_D^{20} = +2.25°$.

0.4000 gm. substance in 15 cc. aqueous solution containing 1 gm. borax rotated in a 2 dm. tube +1.18° to the right; hence $[\alpha]_D^{20} = +22.09°$.

The melting point and optical rotation of this alcohol agree so closely with the physical constants recorded for volemite,[4] that the identity of the two substances might be suspected except for one discordant fact. The melting point of the benzal derivative of volemite is given by Bougault and Allard[4] as 90°, which does not agree with the melting point (200°, see below) for the benzal compound of the heptitol obtained from the sugar sirup. For the present, therefore, the alcohol will be designated as *α-sedoheptitol.*

Tribenzal-α-Sedoheptitol.—3 gm. of α-sedoheptitol were dissolved in 4 cc. of 50 per cent sulfuric acid, 4 gm. of benzaldehyde

[4] Fischer (*Ber. chem. Ges.*, 1895, xxviii, 1973) gives 149–151° as the melting point of volemite. Bougault, J., and Allard, G. (*Compt. rend. Acad.*, 1902, cxxxv. 796), record for volemite $[\alpha]_D$ in water + 1.92° and +2.65°, and in borax solution +20.83°.

were added, and the mixture was shaken thoroughly and allowed to stand over night. The crystalline product of the reaction was washed with water and then with alcohol and finally was twice recrystallized from absolute alcohol. It melted sharply and without decomposition at 199–200° (uncorrected). Analysis shows it to be the tribenzal compound of the heptitol.

0.1227 gm. substance gave 0.3174 gm. CO_2 and 0.0613 gm. H_2O.

	Calculated for $C_{21}H_{26}O_7$:	Found:
C	70.58	70.54
H	5.88	5.55

Tribenzal-β-Sedoheptitol.—The sirupy residue from the α-heptitol, together with the alcoholic washings, was dissolved in water and the solution was concentrated to a sirup. This was dissolved in an equal volume of 70 per cent sulfuric acid in a small glass-stoppered Erlenmeyer flask, and the same volume of benzaldehyde was added. On vigorous shaking crystallization began at once and after 1 hour the contents of the flask had changed to a semisolid mass of crystals. After standing over night these were thoroughly washed with water, filtered off, and then washed again with alcohol. The product was finely ground and was then boiled out several times with alcohol and twice with methyl ethyl ketone. It then melted at about 225–230° but was probably not pure as it may have contained a little of the benzal compound of the α-heptitol and perhaps other substances. It was, however, used in this condition for hydrolysis. For analysis a small part was recrystallized by dissolving in a large volume of hot methyl ethyl ketone, in which it is rather sparingly soluble, and concentrating the solution to a small volume. In all other reagents it is nearly insoluble. It separates from methyl ethyl ketone in tufts of flexible needles resembling asbestos. It melts at 272–275° (uncorrected) without decomposition and crystallizes again on cooling. Analysis shows it to be the tribenzal derivative of a second heptitol which will be named *β-sedoheptitol.*

0.1418 gm. substance gave 0.3646 gm. CO_2 and 0.0727 gm. H_2O.
0.1291 " " " 0.3320 " " " 0.0663 " "

	Calculated for $C_{21}H_{26}O_7$:	Found:	
C	70.58	70.11	70.13
H	5.88	5.69	5.70

β-Sedoheptitol.—15 gm. of tribenzal-β-sedoheptitol (m. p. 225°), which had been purified as described above by extraction with alcohol and methyl ethyl ketone, were finely powdered and boiled for about 12 hours under reflux with 250 cc. of 8 per cent sulfuric acid. When hydrolysis was complete the benzaldehyde was removed with ether and the sulfuric acid with barium carbonate. The solution yielded on evaporation a colorless sirup which crystallized after a few days standing. The mass of crystals was washed with a little 95 per cent alcohol and recrystallized by dissolving in a little water and adding hot absolute alcohol to a faint turbidity. The substance crystallized in short thick prisms which melted at 127–128° (uncorrected), and two additional recrystallizations did not change this melting point. The yield was 6 gm. The compound appears to be optically inactive, since 0.5084 gm. of substance in 5 cc. of water showed no appreciable rotation in a 1 dm. tube. The same amount of substance in 5 cc. of saturated aqueous borax solution showed a rotation of not more than +0.1°. A 20 per cent solution in saturated aqueous borax solution of material three times recrystallized showed a rotation not greater than 0.03°, from which it may safely be concluded that the substance is optically inactive. The analysis agrees with the formula of a heptitol.

0.1651 gm. substance gave 0.2390 gm. CO_2 and 0.1097 gm. H_2O.

	Calculated for $C_7H_{16}O_7$:	Found:
C	39.62	39.47
H	7.54	7.38

The hydrolysis of the benzal compound can also be effected by means of acetic acid, and since much less time is required by this method it is perhaps to be preferred to the one just described. The benzal compound was boiled with 60 per cent acetic acid under reflux until all of it had dissolved and then the solution was distilled under reduced pressure to a sirup. This was diluted with water, the remaining benzaldehyde extracted with ether, and the aqueous solution concentrated to a sirup. This crystallized readily on seeding.

A Benzal Compound from the Transformed Sirup (Dibenzal-Anhydro-Sedoheptose).

It has already been stated that when the sirup containing sedoheptose is treated with dilute hydrochloric acid, a transformation takes place which is indicated by a marked loss in its power of reducing Fehling's solution and by the development of a strong levorotation.

In order to isolate the product of this reaction the sugar sirup was treated with benzaldehyde and sulfuric acid in the hope of obtaining a crystalline benzal compound. It was found that such a derivative could indeed be prepared either from the sirup obtained by evaporating the solution of the transformed sirup or equally well from the crude sirup itself; this would not be unexpected because the sulfuric acid that is employed in the preparation of the benzal compound would naturally bring about the transformation.

60 gm. of crude sirup were mixed with an equal weight of 70 per cent sulfuric acid in a glass-stoppered Erlenmeyer flask at room temperature and 40 gm. of benzaldehyde were added. The mixture was shaken vigorously from time to time throughout the day. Crystals began to appear after 1 to 2 hours, and after 12 hours the contents of the flask had changed to a semisolid crystalline mass. After standing at least 18 hours the mass was thoroughly broken up, filtered off, and washed successively with water, cold glacial acetic acid, and alcohol. The yield was 22 gm. In another experiment, where purified sirup was used, 15 gm. yielded 7.5 gm. In this case the material had been heated in 10 per cent solution with 1 per cent hydrochloric acid and the latter then removed with silver carbonate. In a third experiment, 20 gm. of purified sirup, without previous treatment with hydrochloric acid, yielded 9 gm. of the compound.

The substance crystallizes in prismatic needles which are practically insoluble in all the usual reagents except hot acetic acid and hot acetic anhydride. Acetic acid, however, causes some decomposition. It was recovered unchanged from ten to thirty parts of hot acetic anhydride in beautiful white prisms which often attained a length of 1 cm. 22 gm. of crude material gave 20 gm. of pure substance which melted at 245° (uncorrected).

Analysis and molecular weight determination indicate that the compound has the formula $C_{21}H_{20}O_6$.

0.1380 gm. substance gave 0.3448 gm. CO_2 and 0.0665 gm. H_2O.
0.1429 " " " 0.3586 " " " 0.0696 " "

	Calculated for $C_{21}H_{20}O_6$:	Found:	
C	68.47	68.13	68.43
H	5.43	5.33	5.20

The molecular weight (M) was determined by the boiling point method, using acetic anhydride as a solvent. As K for acetic anhydride was not known this was first determined, using benzil as the standard, and was found to be about 60, as an average of a large number of determinations.

Determination of M (Calculated 368).

$$\text{I.}\quad M = 60\frac{0.2703 \times 100}{21.87 \times 0.235} = 315$$

$$\text{II.}\quad M = 60\frac{0.5470 \times 100}{21.87 \times 0.335} = 445$$

$$\text{III.}\quad M = 60\frac{0.8028 \times 100}{21.87 \times 0.625} = 350$$

$$\text{IV.}\quad M = 60\frac{0.4867 \times 100}{22.78 \times 0.402} = 318$$

$$\text{V.}\quad M = 60\frac{0.7727 \times 100}{22.78 \times 0.542} = 375$$

The formula $C_{21}H_{20}O_6$ agrees for the dibenzal derivative of a compound having the formula $C_7H_{12}O_6$; *i.e.*, a heptose ($C_7H_{14}O_7$) minus a molecule of H_2O.

Since the sugar itself before treatment with acids behaves normally when reduced with sodium amalgam and gives a heptose osazone of normal composition, it is not possible that it could have any other formula than $C_7H_{14}O_7$. It appears therefore that this normal heptose upon treatment with acids loses the elements of H_2O from its molecule and is converted into an anhydride. By hydrolysis of the benzal compound it was indeed found possible to isolate such an anhydride in pure crystalline condition.

Anhydro-Sedoheptose.

25 gm. of the dibenzal compound just described, of the formula $C_{21}H_{20}O_6$, were suspended in 150 cc. of 60 per cent acetic acid

and boiled under reflux. Complete solution took place after ½ hour. The heating was continued for 30 minutes longer, after which the solvents and the greater part of the benzaldehyde were removed by distillation under diminished pressure. The resulting sirup was taken up in water, extracted with ether, and again evaporated as before. This sirup, which was quite colorless, reduced Fehling's solution and from it a small amount of phenylosazone could be obtained. It crystallized after several days standing. The substance after washing with 95 per cent alcohol was recrystallized from the same solvent and yielded short, thick, well formed, colorless crystals which sometimes attained the length of 0.5 cm. It melted at 155° (uncorrected).

$$[\alpha]_D^{20} = \frac{-6.02^\circ \times 5.2149}{1 \times 1.020 \times 0.2149} = -143.0^\circ.$$

The yield of the first product was 9.6 gm. while 0.3 gm. crystallized from the first mother liquor.

The same substance may be obtained directly from the transformed purified sirup if crystals for seeding are available. 35 gm. of purified sirup in 160 cc. of 1 per cent hydrochloric[5] acid were heated for 1 hour on the steam bath. The solution rotated in a 2 dm. tube −28.6°, and as calculated from the specific rotation given above contained about 16 gm. of the anhydride. The hydrochloric acid was removed from the solution with silver carbonate and the slight excess of silver with hydrogen sulfide. The sirup obtained on concentration under diminished pressure was seeded with crystals obtained from the above experiment and yielded after several days standing 8 gm. of the same substance, or 50 per cent of the amount present. It was separated from the thick sirup by diluting with very little 95 per cent alcohol, filtering off the crystals, and washing with the same solvent. It was recrystallized from 95 per cent alcohol and melted at 155° (uncorrected) without decomposition.

[5] Since the sirup generally contained a small amount of barium salts, it must be neutralized to Congo before adding the required amount of hydrochloric acid.

0.1554 gm. substance gave 0.2514 gm. CO_2 and 0.0852 gm. H_2O.
0.1677 " " " 0.2680 " " " 0.0917 " "

	Calculated for $C_7H_{12}O_6$:	Found:	
C	43.75	44.14	43.58
H	6.21	6.09	6.08

0.2870 gm. substance depressed the freezing point of 17.35 gm. H_2O 0.180°.

	Calculated for $C_7H_{12}O_6$:	Found:
M	192	175

The compound will be named *anhydro-sedoheptose.*

Properties.—It showed a levorotation in aqueous solution as follows:

2.2799 gm. substance in 25 cc. H_2O at 20° in a 4 dm. tube rotated 53.39° to the left; hence $[\alpha]_D^{20} = -146.3°$. This value was more accurately determined than the previous one and is accepted as the correct specific rotation.

No mutarotation was observed. The substance is of neutral reaction and possesses a sweet taste. It is only slightly soluble in cold 95 per cent alcohol, fairly easily in the hot solvent, and very easily in cold water. It gives a strong test with orcin and hydrochloric acid. It shows no reduction of Fehling's solution, but after warming with dilute hydrochloric acid the solution becomes reducing. When 0.2335 gm. of the non-reducing anhydro compound dissolved in 20 cc. of 1 per cent hydrochloric acid was heated for 30 minutes on the steam bath in a closed vessel, the amount of reducing substance produced corresponded to 0.0859 gm. of Cu_2O (= 0.0429 gm. of glucose). A second experiment in which 0.2335 gm. was used showed a reducing power after the same treatment which corresponded to 0.0865 gm. of Cu_2O (= 0.0432 gm. of glucose). Referring back to where the action of hydrochloric acid on the sugar sirup was discussed, it was stated there that this reagent caused a change in the power of the sugar sirup to reduce Fehling's solution and that the amount of reducing power of a solution so treated was equal to about 20 per cent of that which the original solution possessed. From the experiment just described, it will be apparent that from a given amount of the non-reducing anhydro-sedoheptose an amount of reducing substance is developed on treatment with 1 per cent hydrochloric acid which, calculated as glucose, is equal to about 18.4 per cent of the amount of anhydride taken. Thus it would seem that in dilute acids about the same condition of equilibrium is reached

between the reducing and non-reducing substances (sedoheptose and anhydro-sedoheptose, respectively), regardless of which one was originally present. It is thus clear that the product obtained by cleavage of the benzal compound of anhydro-sedoheptose with acids was a solution containing both substances in the above proportions, from which a corresponding amount of phenylosazone could be obtained. Since bromine was without action on such a mixture it may be safely concluded that the reducing compound present (sedoheptose) is of the nature of a ketose. It may be assumed also that the oxygen atom of the carbonyl group is involved in the process of elimination of water from the original sugar molecule and that this is the cause of the disappearance of its power to reduce Fehling's solution. Since the heptoses ($C_7H_{14}O_7$) contain six reactive hydroxyl groups, as shown by the fact that the fully acetylated derivative of α-glucoheptose is a hexacetate, it is to be expected that an anhydroheptose ($C_7H_{12}O_6$) would contain two less; *i.e.*, four. This corresponds to the fact that anhydro-sedoheptose yields a dibenzal derivative which can be boiled with acetic anhydride without the occurrence of any acetylation, showing that no reactive hydroxyl groups remain.

SUMMARY.

An aqueous extract of the leaves and stems of one of the stonecrops, *Sedum spectabile*, was found to contain a non-fermentable reducing sugar. The analyses of the crystalline phenyl and bromophenyl osazones that were obtained from it show that the sugar is a new heptose and it has been named *sedoheptose*. From its osone a crystalline compound with *o*-phenylenediamine was obtained. By reduction of a solution of the sugar from the plant extract with sodium amalgam two heptahydroxy alcohols were produced, designated as α- and *β-sedoheptitol*. They were obtained in pure crystalline condition and likewise their benzal derivatives. On heating the plant extract with dilute acid it lost about 80 per cent of its reducing power towards Fehling's solution. When the extract that had been heated with acid was treated with benzaldehyde a crystalline compound was obtained which proved to be the benzal derivative of a heptose that had lost one molecule of water. By hydrolysis of this benzal com-

pound the anhydride was obtained as crystalline *anhydro-sedoheptose*. On heating the latter with dilute acid it passed to the extent of about 20 per cent into sedoheptose, and there is thus an equilibrium in such solutions between the two substances. Sedoheptose is in all probability a ketose, as bromine does not oxidize it and the two alcohols mentioned above probably result from its reduction.

THE CONCENTRATION OF DEXTROSE IN THE TISSUES OF NORMAL AND DIABETIC ANIMALS.

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In the investigation of the fate of dextrose in the animal organism little attention has been given to the amount of this substance existing in the tissues. The object of this paper is to present the results of a study of the distribution and concentration of dextrose or copper-reducing substances[1] in normal and diabetic animals under various conditions. Such an investigation is of importance because of the lack of reliable data in the literature, the possible bearing on the increase during fasting in the respiratory quotient of individuals with diabetes mellitus, the fact that certain diabetics apparently neither excrete nor burn large amounts of carbohydrate taken in the form of oatmeal or other starchy foods, and also because of the possible difference between normal and diabetic organisms in their ability to hold carbohydrate in the form of dextrose in their tissues.

LITERATURE.

In 1853 Claude Bernard (1) published a large number of analyses of liver tissues for dextrose from a variety of mammals, birds, reptiles, and fish. He found in mammals percentages varying from 1 to 4 per cent in all cases when estimations were possible. These tissues were extracted with water

[1] It is appreciated that the copper-reducing power of animal tissue extracts is not due solely to dextrose, but that such substances as creatine, creatinine, uric acid, etc., may play a part. On the other hand, there are substances which may inhibit copper reduction. As the method employed in estimating the reducing power of the tissue extracts was such as to reduce the error caused by these substances to a minimum, the main conclusions are probably little affected by them. The use of the word "dextrose" in connection with its concentration in body tissues signifies the total reducing power of the extract under the conditions imposed.

and enzyme action was stopped by boiling, but in most instances the tissues were not extracted until several hours after the death of the animal. Several years later lower values, 0.08 to 0.44 per cent, for dextrose in the liver were reported by Dalton (2), who appreciated the fact that the concentration of sugar in the liver increased rapidly after the death of the animal and that this increase was due to the hydrolysis of glycogen. By thrusting the livers of well nourished rabbits taken before death into boiling water von Mering (3) found only traces of dextrose. Concentrations of 0.46 to 0.55 per cent in dogs, and 0.56 to 0.60 per cent in rabbit livers were found by Seegen and Kratschmer (4). The work of these investigators was criticized by Girard (5) on the ground that too much time elapsed before the tissues were put into boiling water, also that the tissues contained too much blood. By working quickly, pressing out all possible blood, and extracting with boiling water, he failed to find more than 0.05 per cent dextrose in dog liver.

By far the largest number of tissue analyses for dextrose, 200 or more, have been made by Pavy (6). He used the reliable technique of extracting in boiling water or cold alcohol, and his results include all the important tissues in a great variety of normal animals. Unfortunately the data do not include the blood sugar of the animals from which the various tissues were taken. Pavy considered 0.2 to 0.3 per cent the amount of dextrose ordinarily found in normal dog liver, although he reports values as high as 1.9 per cent. In rabbit livers he found 0.11 to 0.26 per cent. Other tissues of the dog reported are muscle 0.2 to 0.4 per cent, spleen 0.16 to 0.20 per cent, kidney 0.11 to 0.17 per cent, pancreas 0.08 to 0.23 per cent; lungs 0.09 to 0.32 per cent, and brain 0.07 to 0.13 per cent. Butte's (7) experiments led him to the conclusion that normally there was no sugar in the muscles of dogs, but in 30 minutes after an intravenous sugar injection of 4 gm. per kilo he found as much as 0.42 per cent. Bang (8) found 0.06, 0.07, and 0.10 per cent dextrose in the muscles of normal rabbits.

Very few analyses have been made except in normal animals. Hédon (9) reports a few experiments in which the liver was analyzed for dextrose following extirpation of the pancreas in dogs. His values vary between 0.3 and 1.28 per cent, which cannot be considered reliable because he frequently took 5 to 10 minutes in bleeding the animal before taking tissues. The kidneys of six phlorhizinized dogs were found by Levene (10) to have 0.22, 0.22, 0.20, 0.55, 0.15, and 0.28 per cent of dextrose respectively. In dogs that had been made glycogen-free Embden (11) found only traces of dextrose in the liver. Bang (8) reports six experiments performed with starving rabbits in which 2 to 5 gm. of dextrose were given intravenously. The percentage of dextrose in the liver shortly after the end of the injection was found to be considerably higher than that of the blood. In animals with blood sugar of 0.22, 0.20, 0.46, 1.10, and 0.40 per cent the liver contained 0.61, 0.80, 0.85, 1.12, and 0.94 per cent dextrose respectively. Albertoni and Monetti (12) report no dextrose found in muscles of dogs and rabbits. Kleiner (13) has reported analyses of muscles of dogs, in which the aorta and vena cava had been ligated near the diaphragm, before and after intra-

venous injection of dextrose. In his experiments ether and morphia were used as anesthetics, 4 gm. of dextrose in 20 per cent solution allowed to run into the vein at the rate of about 3 cc. per minute, and the animal was killed 1½ to 2 hours after the end of the injection. Three such experiments are reported. Blood sugars just before injection were 0.33, 0.15, and 0.17 per cent, muscle sugars before injection 0.26, 0.40, and 0.52 per cent, after injection 0.36, 0.71, and 0.80 per cent respectively. He also found a considerable increase in the reducing power of the muscle extracts after boiling with 1.8 per cent hydrochloric acid, especially after injection of sugar. This was most marked when sugar was injected into dead animals. In three of his experiments the increase amounts to as much as the sugar found before hydrolysis. The muscle tissue after being passed through a meat chopper was extracted with water under toluene for 20 hours in the ice chest. After filtering through cheese-cloth a little acetic acid was added to the extract, which was then boiled and filtered through glass wool, the final clearing of proteins being accomplished by phosphotungstic acid.

From the foregoing review of the literature, a wide variation is seen to exist in the amounts of dextrose found in animal tissues. With the exception of Pavy's (6), most work has been done with the liver, which in dogs is credited with concentrations of dextrose varying between traces and 4 per cent, while in rabbits amounts from mere traces to 0.26 per cent are given. In dogs the muscle sugar values vary between traces and 0.40 per cent. The amounts found in rabbit muscles vary between traces and 0.10 per cent. The concentrations of dextrose in spleen, pancreas, kidney, lungs, and brain, as given by Pavy, range between 0.08 and 0.13 per cent with little difference between the several organs.

The chief criticism of the work on dextrose in animal tissues is the unreliable technique employed. In most instances the rapidity with which hydrolysis of glycogen may occur did not seem to be appreciated. Only a few workers have included the blood sugar values of the experimental animals. Also, comparisons between diabetic and non-diabetic conditions have never been made.

Analyses of human tissues, both normal and diabetic, were made by Griesinger (14), but the tissues were taken several hours after death so that the results are of little value.

EXPERIMENTAL.

Much experimentation was carried out to determine the most efficient and reliable method for extraction of dextrose from animal tissues. A very essential detail which has apparently been overlooked in previous work is the prevention of enzyme action at the time of taking tissues for analysis. Boiling water and boiling sodium sulfate solution acidified with acetic acid, boiling and cold (−5–10°) alcohol, as well as the use of liquid air

were all tried as methods of preventing enzyme action and also to test their relative practicability. Boiling water kills very quickly all enzymes found in the several tissues, while alcohol, especially cold alcohol, and liquid air merely inhibit their action so that the danger in future manipulation of getting some enzyme action is not inconsiderable. Acid mixtures were discarded because of the danger of possible hydrolysis of higher sugars and sugar compounds.

Tissues ground in a meat chopper before extraction were found to increase the sugar content to double or treble the amount found if enzyme action were stopped immediately on removal of the tissues from the body, thus prohibiting this procedure. Extraction in the cold with water proved unsatisfactory because enzyme action was not wholly inhibited by temperature above 0°C.

Boiling water proved to be more efficient than alcohol in checking enzyme action; hence it was adopted. The difficulty in obtaining and manipulating liquid air, with its slight superiority over boiling water, led us to discard its use.

The manner in which protein could be removed from the extracts was the subject of considerable investigation. A distinct advantage offered by alcohol is that the protein is taken out in the process of extraction, while in salt or acidified solutions only a portion of the protein is taken out, necessitating a second procedure for its complete removal. After experimenting with meta-phosphoric acid, phosphotungstic acid, hydrochloric acid, and mercuric chloride (Schenk's method), aluminium hydroxide, and kaolin, colloidal iron (Michaelis and Rona (30)) was found to be the most convenient and satisfactory.

Method.

The experiments were performed on dogs and rabbits. Except in two or three particular instances anesthesia of any kind was avoided because of the well known effect on blood sugar and the possible alterations in the various tissues which might follow. In rabbits, intravenous injections were made in the ear vein, and blood samples were taken from the heart. In dogs, intravenous injections were made and blood samples taken from the external jugular vein by means of a Luer syringe. Before taking tissues

for analysis all animals were stunned by a blow on the head, and, with the exception of a few animals which were bled from the carotid for 3 or 4 minutes, the tissues were immediately weighed on an Eimer and Amend No. 330 triple beam balance and thrust immediately into boiling water. The sample was then cut into small pieces in the boiling water, boiling being continued for at least 15 minutes. At the end of this time the beakers were placed on a boiling water bath. As soon as convenient the water covering the tissue was filtered through glass wool and the tissue finely divided with a macerator. A second extraction on the water bath for a period of 1 hour was made, and finally three such extractions of 10 minutes each, making altogether five water extractions. The combined extracts which had been filtered through glass wool were measured (usually about 700 to 800 cc. for 50 gm. of tissue) and the protein was removed by colloidal iron (Merck's 5 per cent). It was found that muscle extracts require 2 cc. per gm. of tissue, while all other extracts cleared perfectly with 1 cc. of colloidal iron per 1 gm. of substance. The iron is added slowly with constant stirring or shaking, 5 to 10 cc. of a 40 per cent sodium sulfate (crystals) solution are added in a similar manner and placed on the water bath for an hour, and then filtered.[2]

The entire or an aliquot part of the filtrate is concentrated to small volume on the water bath. All liver extracts, however, were concentrated *in vacuo* because of the tendency of these extracts to become highly colored when brought to a small volume in the water bath. Benedict's (15) titration method for sugar estimation was used because creatine, uric acid, etc., cause less error than in Fehling's solution. Frequently only small amounts of material were available, so 10 cc. instead of 25 cc. of the copper solution were used and in instances where there was not sufficient to reduce the entire amount of copper the titration was finished by adding the necessary amount of a 0.2 per cent dextrose solution. Blood sugar was determined by the method of Lewis and Benedict (16).

[2] Both sodium sulfate and heating are not absolutely essential, but we found that they facilitated filtration considerably. The use of colloidal iron for removal of protein from solutions containing dextrose was suggested by Michaelis and Rona (30).

Normal Animals.

Dogs were taken from the stock supply (ordinary diet, bread and soup) and on the day of the experiment received no food. The results of the analyses are collected in Table I.

TABLE I.

Normal Dogs.

No. of experiment.	Blood sugar.		Dextrose in tissues.										
	Whole blood.	Plasma.	Liver.	Skeletal muscle.	Heart muscle.	Kidney.	Spleen.	Pancreas.	Stomach.	Small intestine.	Skin.	Lungs.	Brain.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
N IV	0.10	0.11	0.26	0.07	0.20				0.04				
			(0.27)*	(0.10)*	(0.25)*				(0.04)*				
342**	0.10	0.10	0.47	0.04	0.07	Trace.	0.06	0.06	0.03	0.02		0.01	0.01
341**	0.11	0.12	0.22	0.07	0.02	0.05	0.02		0.01	0.03	0.01	0.01	0.02
343**	0.11	0.13	0.43	0.07		0.05	0.03	Trace.	0.02	0.01	0.02	0.04	0.01
316†	0.13	0.10	0.14	0.09	0.07	0.07	0.07	0.11	0.04	0.04	Trace.	0.06	Trace.
321‡				0.07	0.05	0.07							0.05

* After hydrolyzing extracts with 1.8 per cent HCl, and boiling for 1½ hours with a reflux condenser.

** Animals were bled before taking of tissues.

† Dog proved to have a tumor of the ovary. Tissues in addition to those in the table showed: bladder 0.04 per cent; parotid 0.04 per cent; esophagus 0.03 per cent; ovarian tumor 0.03 per cent; tongue 0.02 per cent; thyroid, aorta, and trachea only traces.

‡ Died under ether anesthesia; tissues were taken within 10 minutes.

The blood sugar in these animals varied between 0.10 and 0.13 per cent, averaging 0.11 per cent, which is within the range usually given for the normal blood sugar values in dogs (16). A wide variation in the liver dextrose is at once noticeable, varying from 0.14 to 0.47 per cent, making any average of the several observations of little significance. The concentration of dextrose in the muscles is well under that found in the blood or liver and is much more constant, varying from 0.04 to 0.09 per cent, averaging 0.07 per cent. The heart muscle with one exception, N IV, seldom contained more sugar than did the skeletal muscle. In the other tissues, kidneys, spleen, pancreas, stomach, intestine, skin, tongue,

thyroid, and brain, the amount of dextrose present is small and with the exception of the pancreas in Dog 316 invariably lower than in the muscle. All of these tissues taken together, of course, amount to much less than the muscle, which is usually considered to comprise three-sevenths of the body weight. In Dog N IV a slight increase in the dextrose in the muscle extracts is found after boiling with hydrochloric acid. The skeletal muscle increased from 0.07 to 0.10 per cent, the heart muscle from 0.20 to 0.25 per cent, but no increase was found in either liver or stomach.

Effect of the Duration of Hyperglycemia.

Before making observations on animals in which dextrose had been administered to determine the amount of sugar the several

TABLE II.

Effect of the Duration of Hyperglycemia on the Concentration of Dextrose in Normal Rabbits.

No. of experiment.	Dextrose in plasma before sugar injection.	Dextrose in blood immediately before killing.		Dextrose in tissues.			Remarks.
		Whole blood.	Plasma.	Liver.	Muscle.	Stomach.	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
I	0.13			0.09			Received no dextrose.
II	0.17			0.17			" " "
III	0.16	0.35	0.50	0.37	0.11	0.12	Received 8 gm. of dextrose per kilo subcutaneously; killed 2½ hrs. after sugar injection.
IV	0.13	0.44	0.50	0.42	0.08	0.12	Received 8 gm. of dextrose per kilo at beginning of experiment; 8 hrs. later 4 gm. per kilo given subcutaneously. Killed 12 hrs. after first injection.
V	0.20	0.42	0.49	0.44	0.04	0.21	Received 8 gm. of dextrose per kilo at beginning; 12 hrs. later 4 gm. per kilo and 22 hrs. later 4 gm. per kilo. All sugar was given subcutaneously. Killed 24 hrs. after first injection.

Merck's anhydrous dextrose was used in all experiments including the use of dextrose.

tissues had the power of taking up, experiments were performed to see what effect the duration of hyperglycemia might have in this respect. Rabbits were chosen for this purpose. The results are given in Table II.

A hyperglycemia maintained over periods from 2½ to 24 hours had little effect on the amount of sugar that the liver, skeletal muscle, and stomach held as free dextrose.

Effect of Bleeding on Dextrose in the Tissues.

In certain of the experiments a large quantity of blood was desired for special investigations. Hence bleeding from the carotid for 3 to 4 minutes was performed until from an animal weighing between 10 and 15 kilos 400 to 500 cc. of blood were taken. The impression gained from the literature was that glycogenolysis in the liver to any marked extent did not take place for 2 or 3 minutes after the death of the animal. We anticipated that if the tissues were taken before the heart stopped beating there would be no significant change. It soon developed that bleeding had a profound effect on glycogenolysis in the liver. At the same time it came to light that glycogenolysis in the muscles and other tissues did not occur to any significant extent, making it possible to study the effect of bleeding on the liver dextrose and at the same time to use the other tissues for dextrose determinations. The liver tissue was taken immediately after the animal was stunned, thus avoiding the effect of subsequent bleeding.

If the liver tissue be taken immediately (within 10 seconds) after stunning, before bleeding has progressed to any extent, there is apparently little hydrolysis of glycogen, as may be seen in Experiments 351, 355, 352, and 353. Plasma and liver dextrose correspond quite closely except in Dog 350, where the difference is considerable. As the time of taking the specimen increases, so also does the dextrose in the liver tissue until in one instance, Dog 344, when 5 minutes elapsed from the time the animal was stunned, but only 1 minute from the end of the bleeding, before the liver was taken, an increase of the tissue over the plasma sugar amounting to 340 per cent had occurred. When the animals were not bled but tissue was taken soon after stunning, out of five animals in only one did the liver contain much more dextrose than the

plasma. The increase in dextrose in consecutive specimens of liver was found to be greater in the animals which were bled than in those not bled. In diabetic animals, as might be expected when

TABLE III.

Effect of Bleeding on Dextrose in the Liver.

No. of experiment.	Plasma dextrose.	Time between stunning and taking of liver.	Liver dextrose.	Difference between plasma and liver.
		Normal animals bled.		
	per cent		*per cent*	*per cent*
350	0.27	10 sec.	0.37	+0.10
351	0.28	10 "	0.28	0.00
355	0.83	10 "	0.75	−0.08
352	1.06	10 "	0.75	−0.31
353	0.08	1 min.	0.08	0.00
347	0.19	2 "	0.85	+0.66
342	0.10	4 "	0.48	+0.38
341	0.12	5 "	0.22	+0.10
344	0.13	5 "	0.58	+0.45
343	0.13	6 "	0.43	+0.30
340	0.21	7 "	0.80	+0.59
		Normal animals without bleeding.		
315	0.55	10 sec.	0.47	−0.08
317	0.27	1 min.	0.32	+0.05
316	0.10	1 "	0.14	+0.04
297	0.68	2 "	0.65	−0 03
325	0.26	3 "	0.47	+0.21
		Diabetic animals bled.		
302	0.53	4 min.	0.34	−0.19
319	0.44	5 "	0.20	−0.24
322	0.56	7 "	0.38	−0.18
328	0.71	8 "	0.35	−0.36
		Diabetic animals without bleeding.		
280	0.26	10 sec.	0.26	0.00
271	1.00	1 min.	0.54	−0.46
287	0.29	1 "	0.16	−0.13
320	0.58	7 "	0.49	−0.09

glycogen in the liver exists in small amounts or is absent, the liver dextrose is invariably lower than the plasma. Glycogenolysis in muscles is much slower than in the liver. This was shown by repeated analyses of muscle samples taken at intervals during 15 minutes after stunning. The changes were always insignificant.

Intravenous Dextrose in Rabbits.

Bang (8) has reported a series of experiments on normal fasting rabbits in which following an intravenous injection of dextrose there was a higher concentration of sugar in the liver than in the blood. This observation seemed of sufficient importance to confirm because of its bearing on glycogenesis and its possible significance in diabetes. Accordingly Bang's experiments were repeated. Normal rabbits which had been fasted for 6 days were used (Table IV).

TABLE IV.

Intravenous Dextrose in Rabbits. Effect on Dextrose in Liver.

No. of experiment	VI.	VII.	VIII.	IX.	X.	XI.
Weight, *gm*	1,750	2,000	2,750	2,175	2,125	1,925
Amount of dextrose injected, *gm*	2.0	2.0	4.0	4.0	5.0	5.0
Time for injection, *min*	13	13	15	15	21	30
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Blood sugar before injection	0.14	0.13	0.13	0.15	0.13	0.15
" " after "	0.38	0.41	0.56	0.72	0.67	1.06
Plasma " " "	0.56	0.54	0.72	0.93	0.98	1.47
Liver dextrose	0.40	0.41	0.54	0.66	0.67	0.72
Muscle "	0.21	0.12	0.17	0.18	0.16	0.20
Liver glycogen	Trace.	Trace.	0.25	0.31	0.53	1.03
Muscle "	"	"	0.08	0.10	0.15	0.13

We were not able to confirm Bang's observations, for in all six rabbits the concentration of sugar in the liver was below that in the plasma, and also below the whole blood values except in Rabbit VI, where the difference amounts to 0.02 per cent with a whole blood sugar of 0.38 per cent. From a trace in the liver, when 2.0 gm. of dextrose were given, the glycogen increases to 0.53 and 1.03 per cent in the two rabbits receiving 5.0 gm. Both dextrose and glycogen exist in small amounts in the muscles.

Effect of Dextrose Administration in Normal Animals.

Dextrose was given by mouth, subcutaneously, intravenously, and intraperitoneally to determine, in addition to the effect of hyperglycemia itself, whether the manner of administration played any part in the ability of tissues to take up dextrose. That there might be a difference in the behavior of tissues in concentrating sugar was suggested by the work of Allen (17) who found that after administration of dextrose to normal animals by any route except intravenously, a marked oliguria resulted, whereas sugar given to diabetic animals by any route whatsoever caused a marked diuresis. Allen's interpretation of this phenomenon was that in normal animals the sugar in passing through the various tissues to the blood stream when given by mouth, subcutaneously, or intraperitoneally underwent some change which resulted in its appearance in the blood in a different physiological state than when introduced intravenously. The diuresis, glycosuria, and loss of power of utilization of sugar in diabetes are thought to be due in part to the presence of dextrose in the blood in some abnormal (probably crystalloid) state.

In all the experiments with the exception of Dog I II the attempt was made to kill the animal at the height of the hyperglycemia. Four animals were given levulose by mouth to see if this particular sugar was held in amounts different from dextrose. The results of the several experiments are collected in Table V.

As in all other experiments, the chief interest centers about the dextrose in the muscles and the liver. It will be seen that the amounts of dextrose found in the liver are quite variable. The extreme, and apparently somewhat variable, rapidity with which glycogenolysis occurs makes the liver values less significant than was hoped. When compared with normal values there is an increase in the muscle dextrose as the blood sugar increases, but it is not always directly proportional to it.

In the animals receiving sugar by mouth, having a plasma dextrose of 0.24 and 0.63 per cent, there was 0.11 and 0.21 per cent respectively in the skeletal muscles. When large amounts of sugar were given subcutaneously the plasma sugar varied between 0.25 and 0.40 per cent but the skeletal muscle dextrose never rose above 0.10 per cent. The highest values were obtained after intravenous administration of dextrose. Dog 352 had a plasma sugar

TABLE V.

Normal Dogs. Dextrose by Mouth, Subcutaneously, Intravenously, and Intraperitoneally.

No. of experiment.	Amount of sugar given per kilo.	Time after sugar administration that tissues were taken.	Blood sugar.				Dextrose in tissues.										
			Before sugar administration.		At time the tissues were taken.		Liver.	Skeletal muscle.	Heart muscle.	Kidney.	Spleen.	Pancreas.	Stomach.	Skin.	Lungs.	Brain.	Eyes.
			Whole blood.	Plasma.	Whole blood.	Plasma.											
Dextrose by mouth.																	
	gm.	*hrs.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
315	12	1	0.18	0.20	0.55	0.63	0.47	0.21	0.08			0.12		0.10			
350	7	1	0.09	0.13	0.19	0.24	0.37	0.11	0.12	0.14	0.08	0.08		0.03	0.09	0.05	0.08
							(0.37)*	(0.20)		(0.15)					(0.09)		
Levulose by mouth.**																	
340	12	2½	0.10	0.11	0.19	0.21	0.80	0.06	0.05	0.09	Trace.	0.03		0.05		Trace.	
344	12	2	0.11	0.14	0.13		0.58	0.07	0.12	0.18	0.08	0.25		0.18	0.12	0.03	0.07
347	12	2	0.10	0.11		0.19	0.86	0.11	0.13	0.17	0.08	0.10		0.14	0.14	0.04	0.08
							(0.85)	(0.16)	(0.17)	(0.17)	(0.09)	(0.14)		(0.14)	(0.14)	(0.06)	
351	12	2		0.10		0.17	0.28	0.12									

Dextrose subcutaneously.

317	10	$2\frac{3}{4}$	0.09	0.09	0.20	0.27	0.32	0.10	0.22	0.20***	0.07	0.04	0.08			0.02	
333†	10	4	0.10	0.12	0.22	0.25		0.09	0.29	0.18***	0.05	0.06	0.07	0.10	0.09	0.06	
337‡	10	3	0.12	0.13	0.27	0.40	0.62	0.10	0.08	0.19§	0.05	0.07	0.08	0.03		0.03	

Dextrose intravenously.

352	55	(6 min.)		0.13		1.05	0.75	0.41	0.56				0.52	0.45	0.50	0.11	0.17
							(0.77)	(0.46)	(0.61)				(0.48)	(0.46)	(0.54)		
I II	4	$1\frac{1}{2}$	0.16	0.17	0.20	0.25	0.56	0.27	0.31				0.13				0.17
							(0.58)	(0.35)	(0.35)				(0.16)				

Intraperitoneal dextrose.

355	8	1		0.10		0.83	0.75	0.33	0.33				0.46		0.34		
							(0.72)	(0.32)	(0.36)				(0.46)		(0.34)		

* All values enclosed in parentheses are those obtained after hydrolysis with 1.8 per cent hydrochloric acid by boiling $1\frac{1}{2}$ hours with a reflux condenser.

** Levulose obtained from Schering and Glatz contained 91 per cent levulose and 9 per cent dextrose. All animals receiving levulose were bled before taking tissues. The time of bleeding did not exceed 4 minutes.

*** Urine contained about 5.0 per cent dextrose, which probably accounts for the high percentage of dextrose found in the kidney tissue.

† Tissues in addition to those in the table: tongue 0.11 per cent; œsophagus 0.18 per cent; bladder 0.24 per cent; thyroid 0.03 per cent; aorta 0.13 per cent.

‡ Animal bled to death.

§ Urine contained 1.0 per cent dextrose.

of 1.05 per cent and a skeletal muscle sugar of 0.41 per cent. The sugar in heart muscle varies in much the same manner as it does in skeletal muscle and seems to have about the same concentration. Little comment is necessary concerning the various other tissues examined. Except when sugar was given by mouth the stomach was analyzed for sugar for the purpose of comparing smooth with striated muscle. Here again the higher the blood sugar the greater the amount of dextrose found in the tissue. No appreciable difference is to be noted between the effects of levulose and glucose in the concentration of sugar in the liver, muscles, or other tissues.

In several of the experiments the extracts of some of the tissues were hydrolyzed with hydrochloric acid. Only the extracts of striated muscle showed any increase in reducing power after boiling with hydrochloric acid.

Influence of Adrenalin.

Adrenalin was used to produce a hyperglycemia in normal animals to determine the effect of glycogenolysis on the tissue dextrose (Table VI).

TABLE VI.

Normal Dogs. Effect of Adrenalin.

No. of experiment.	Time after adrenalin that tissues were taken.	Blood sugar before adrenalin.		Blood sugar at time tissues were taken.		Dextrose in tissues.					
		Whole blood.	Plasma.	Whole blood.	Plasma.	Liver.	Skeletal muscle.	Heart muscle.	Stomach.	Skin.	Eyes.
	hrs.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
361*	2		0.12		0.32	0.29	0.25	0.21	0.11		0.13
						(0.27)**	(0.24)	(0.20)	(0.12)		
A II	2¼	0.11	0.11	0.26	0.36	0.52	0.21	0.22	0.14	0.18	0.12
						(0.52)	(0.36)	(0.25)	(0.17)	(0.19)	
A III	2	0.10	0.11	0.24	0.29	0.51	0.14	0.23	0.15	0.17	0.10
						(0.56)	(0.28)	(0.27)	(0.17)		

* Parke, Davis and Company adrenalin solution 1: 1,000, 1 cc. per kilo was used in all these experiments.

** All values enclosed in parentheses are those obtained after hydrolysis with 1.8 per cent hydrochloric acid by boiling for 1½ hours with a reflux condenser.

There seems to be no striking difference between the amount of dextrose in the tissues when hyperglycemia is caused by adrenalin and when it results from the administration of dextrose. There is no elevated concentration in the tissues to indicate glycogenolysis or the pouring of dextrose by the cells into the blood. On hydrolysis the only extracts yielding extra sugar are those of skeletal muscles. In Dog A III, with a skeletal muscle sugar of 0.14 per cent, on boiling with acid the concentration is increased to 0.28 per cent.

Effect of Phlorhizin and Adrenalin.

An animal was phlorhizinized to see if any effect on the tissue sugar resulted from the rapid loss of glycogen without hyperglycemia. In two other dogs phlorhizin and adrenalin were given as recommended by Sansum and Woodyatt (18) to secure glycogen-free animals for the administration of dextrose. The purpose of these experiments was to see if the presence of glycogen in muscle bears any relation to the sugar in the tissue before and after hydrolysis. Table VII contains the results of these experiments.

It is not surprising to find in the phlorhizinized animal, No. 353, low values for dextrose in the tissues. With a decrease in glycogen the possibility of sugar increase as the result of rapid glycogenolysis during extraction is ruled out. The liver dextrose amounting to only 0.08 per cent is the lowest value found in any of the animals. No marked difference from the normal animals is noted in the thigh muscle, heart, or stomach sugar. An increase from 0.08 to 0.12 per cent in the skeletal muscle extract was found after hydrolysis. There is no marked difference between the concentration of dextrose in the tissues of Dogs P II and P III and other animals not free from glycogen. The liver values approximate those of the plasma, while thigh, heart, and stomach muscle shows amounts near the average usually found when the blood sugar is of the same relative value.

In Dog P II we started with an animal whose muscles were glycogen-free, as shown by analysis before sugar was given, nor was there any glycogen found in either muscles or liver 1½ hours after sugar was given. The dextrose in the muscles before sugar injec-

TABLE VII.

Phlorhizinized Animals.

Experiment.		Blood sugar.				Tissue dextrose.						
		Before sugar injection.		After sugar injection. Just before killing.								
No.	Description.	Whole blood.	Plasma.	Whole blood.	Plasma.	Liver.	Skeletal muscle.	Heart muscle.	Stomach.	Eyes.	Pancreas.	Lungs.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
353	1 gm. of phlorhizin in olive oil subcutaneously 3 days in succession previous to killing for tissues. No sugar given.	0.08	0.08			0.08	0.08 (0.12)*	0.10	0.04	Trace.	0.03 *	0.03
P II**	1 gm. of phlorhizin subcutaneously and 1 cc. adrenalin 1:1,000 subcutaneously every 3 hours (nights excepted) until D:N ratio was constant. 4 gm. per kilo dextrose intravenously; killed 1½ hrs. after.	0.07	0.09	0.26		0.23 (0.27) †	0.13 (0.32) †	0.21 (0.29) †	0.15 (0.19) †	0.12		
P III**	Animal made glycogen-free as in previous experiment, 4 gm. per kilo intravenously; killed in 1½ hrs.	0.06	0.09	0.37	0.47	0.55 (0.57) ‡	0.25 (0.29) ‡	0.22 (0.27) ‡	0.15 (0.20) ‡	0.13		

* Value in parentheses after boiling with 1.8 per cent HCl.

** The sugar injections were made and first specimens of muscle taken under light ether anesthesia.

† Values in parentheses after boiling with 1.8 per cent HCl. Muscle analyzed for glycogen before sugar injection was negative. A trace was found after sugar was given. Liver negative. Sugar in muscle before sugar was given = 0.12 per cent.

‡ Values after hydrolysis are those in parentheses. Muscle analyzed for glycogen before sugar was given was found negative. Sugar content was 0.18 per cent, after hydrolysis 0.19 per cent. Liver glycogen after sugar was 0.163 per cent and muscle glycogen 0.340 per cent.

tion was 0.12 per cent, afterward 0.13 per cent, showing practically no change. However, on hydrolysis of the muscle extract after sugar injection there was an increase from 0.13 to 0.32 per cent or nearly threefold in the sugar values. Hydrolysis of the muscle extracts produced less increase in the sugar values in Dog P III. Starting with practically no hydrolyzable sugar before dextrose was given, 1½ hours afterward the skeletal muscle showed only 0.04 per cent, the heart muscle 0.05 per cent, and the stomach 0.05 per cent increase after boiling with hydrochloric acid. Although in Dog P III no glycogen was found in the muscles before sugar injection, we found 0.16 per cent and 0.34 per cent in liver and muscle respectively after injection.

Diabetic Animals without Sugar Administration.

Various stages and degrees of diabetes[3] are represented in this group of animals. A brief history of the clinical course of the dogs is found in the table giving the results of the tissue analyses (Table VIII).

The striking fact revealed in the tissue analyses of this group of animals is the low concentration of dextrose found in both liver and muscle as compared with the hyperglycemia at the time the tissues were taken. With an average plasma sugar of 0.41 per cent, the average liver sugar was 0.46 per cent, while the muscle sugar averaged only 0.12 per cent, which exceeds but little the skeletal muscle in normal animals in Table I where the plasma sugar average was only 0.11 per cent. The heart muscle is also relatively low in dextrose. In Dog 338 where total pancreatectomy was performed the plasma sugar was 0.71 per cent, the liver was only 0.35 per cent, skeletal muscle 0.14 per cent, and heart muscle 0.10 per cent. The sugar concentration in all other tissues of this series is low. Attention should be called to the fact that the tissues of diabetic animals are low in glycogen. In the livers of Dogs 280 and 322, which were in an extremely weak state, no glycogen was found in either muscles or liver. The

[3] All pancreatectomy operations were performed by Dr. F. M. Allen. Many of the diabetic dogs had been under his observation for several months.

muscle glycogen 0.340 per cent.

TABLE VIII.

Diabetic Animals without Sugar Administration.

No. of experiment.	Description of animal.	Blood sugar.		Tissue dextrose.										Remarks.
		Whole blood.	Plasma.	Liver.	Skeletal muscle.	Heart muscle.	Kidney.	Spleen.	Stomach	Small intestine.	Skin.	Lungs.	Brain.	Eyes.	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
300	4 months previously $\frac{9}{10}$ of pancreas was removed. Would show sugar on bread, not meat, without glycosuria. For several weeks, hyperglycemia.	0.21	0.23	0.59	0.09	0.06	0.06		0.04		0.10		0.03	0.05	
280	$1\frac{1}{2}$ yrs. previously $\frac{4}{5}$ of pancreas was removed. Sugar-free for 1 yr. Then given high carbohydrate diet to impair tolerance. Preceding 6 months gradually lost tolerance; showed sugar on lung and suet; developed coma; died.	0.14	0.26	0.26	0.06	0.04	0.08				0.01		0.03		Liver and muscle contained no glycogen.

320	$\frac{4}{5}$ of pancreas was removed 4 months ago. After 2 months' feeding with bread and soup showed large amounts of sugar. Sugar-free on lung and suet. Heavy glycosuria at death.	0.58		0.49	0.12	0.13	0.37	0.09	0.13	0.08	0.08	0.20	0.03	0.21	
322	$\frac{11}{12}$ of pancreas was removed 6 months ago. Excreted sugar on bread and soup. 1 month before began to emaciate; would not eat; glycosuria.		0.56	0.37	0.26	0.28			0.29						No change in sugar content after boiling with 1.8 per cent HCl. Muscles and liver contained traces only of glycogen.
319	$\frac{5}{6}$ of pancreas was removed 5 months ago. Could not take bread and soup without glycosuria. 1 month excreted sugar on lung diet. Used for several sugar administration experiments. Hyperglycemia without glycosuria.		0.44	0.20	0.02	0.08	0.12		0.12		0.01	0.12	Trace.		Heavy meat diet 3 days before death.

TABLE VIII—*Concluded.*

No. of experiment.	Description of animal.	Blood sugar.		Tissue dextrose.										Remarks.
		Whole blood.	Plasma.	Liver.	Skeletal muscle.	Heart muscle.	Kidney.	Spleen.	Stomach.	Small intestine.	Skin.	Lungs.	Brain.	Eyes.	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
292	About ½ of pancreas was removed 5 months ago; the rest placed under skin. All pedicles cut 2 months ago. Glycosuria developed 1 month ago on lung and suet. Progressive.		0.27	0.83	0.05	0.15	0.10		0.09	0.05	0.06		0.06		Bled before tissues were taken.
338	Total pancreatectomy 24 hrs. before killing. Heavy glycosuria.	0.57	0.71	0.35	0.14	0.10	0.33	0.14	0.15		0.28		0.04		
397	⅝ of pancreas was removed 5 months ago. For past 2 months forced fat and protein diet without glycosuria but increasing hyperglycemia and gain in weight. Gradual coma for past 3 days; dying.		0.38	0.59 (0.59)	0.21 (0.30)										Muscle, glycogen-free. Liver, glycogen a trace. Value in parentheses = after boiling with 1.8 per cent HCl.

tissue extracts in Dog 322 showed no increase in reducing substances on hydrolysis while in Dog 397 the muscle showed an increase of 0.09 per cent with no change in the liver extract.

Effect of Sugar Administration in Diabetic Animals.

Much the same type of animal was used in these experiments as in the preceding group. It comprises mild diabetes, total pancreatectomy, and various intermediate stages (Table IX).

As in the group of diabetic dogs which were given no sugar, the striking feature is the low concentration of dextrose in the liver, thigh, and heart muscles as compared with the degree of hyperglycemia produced. The average plasma sugar was 0.72 per cent, liver 0.55 per cent, skeletal muscle 0.18 per cent, and the heart muscle 0.21 per cent. In normal animals the tissues contained a higher concentration of dextrose when the plasma sugars were comparable. The spleen, stomach, skin, lungs, brain, etc., all contain small amounts of sugar. As the plasma sugar increases there is a corresponding increase in the dextrose values of all these tissues. In Dogs 287, 297, and 309 a great variety of tissues were analyzed. The variations in amounts of dextrose in the several tissues follow quite closely the variations in hyperglycemia. No increase in sugar or hydrolysis is observed in any of the tissues of Dog 271, while in the totally depancreatized Dog D I the muscle extract nearly doubled in reducing power after boiling with acid.

Comparison of Normal and Diabetic Animals.

Both normal and diabetic tissues exhibit a tendency to increase in concentration of dextrose as the sugar in the blood increases. This tendency, however, appears to be much more marked in the normal than in the diabetic animals. To show this relation more clearly Table X has been constructed. The concentrations of dextrose in the skeletal and heart muscle of normal and diabetic animals having nearly comparable blood sugars are placed together.

No marked difference is to be seen in the liver comparisons, but both skeletal and heart muscle show a decidedly lower amount of dextrose in the diabetic than in the normal animals, when the blood sugars are comparable.

TABLE IX.

Diabetic Animals with Sugar Administration.

No. of experiment.	Description of animal.	Blood sugar.				Tissue dextrose.										
		Before sugar.		After sugar and just before death.		Liver.	Skeletal muscle.	Heart muscle.	Kidney.	Spleen.	Stomach.	Small intestine	Skin.	Lungs.	Brain.	Eyes.
		Whole blood.	Plasma.	Whole blood.	Plasma.											
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
302	$\frac{4}{5}$ of pancreas was removed 5 months ago. Tolerance decreased until at time of experiment dog has marked glycosuria on fat and protein diet. Given 1 gm. of dextrose per kilo by mouth; killed 2 hrs. later.		0.39		0.53	0.34	0.05	0.18	0.24		0.12	0.05	0.18	0.16	0.01	0.20
287**	$\frac{4}{5}$ of pancreas was removed 4 months ago. Could take high carbohydrate diet without showing glycosuria. Moderate hyperglycemia. 10 gm. per kilo subcutaneously. Died suddenly 2 hrs. later. Tissues taken.	0.17	0.18	0.27	0.29	0.22	0.05	0.11	0.22	0.09	0.09	0.03	0.06	0.17	0.04	0.10

271	$\frac{5}{6}$ of pancreas was removed $1\frac{1}{2}$ yrs. ago. By feeding, tolerance for sugar gradually lowered until the dog excreted large amounts of sugar on fat and protein diet. 0.5 gm. of dextrose intravenously and 7 gm. of dextrose subcutaneously 4 hrs. before killing.	0.33	0.40		1.00	0.48 (0.48)*	0.33 (0.32)	0.27 (0.27)	0.85 (0.82)	0.28	0.37 (0.37)	0.36 (0.35)	0.56 (0.56)	0.49 (0.45)	0.08	0.39
309***	$\frac{2}{3}$ of pancreas was removed 6 days before experiment. Dog refused to eat; began to decline with heavy glycosuria. Remnant removed at beginning of experiment. 1 gm. of dextrose per kilo intravenously 9 hrs. before death. 1 liter of salt solution 6 hrs. before death. Blood sugar before salt = 1.00 per cent.	0.17	0.20	0.67	0.80	0.83	0.39		0.39	0.34		0.18		0.33	0.10	0.39
325	$\frac{3}{4}$ of pancreas was removed 1 month before; did not do well. $\frac{1}{2}$ hr. before killing $\frac{1}{2}$ gm. of dextrose per kilo intravenously. Urine contained no sugar at beginning of experiment.	0.11	0.15	0.23	0.29	0.40	0.07	0.14	0.07	0.06			0.11		0.04	

TABLE IX—*Concluded.*

No. of experiment.	Description of animal.	Blood sugar.				Tissue dextrose.										
		Before sugar.		After sugar and just before death.		Liver.	Skeletal muscle.	Heart muscle.	Kidney.	Spleen.	Stomach.	Small intestine.	Skin	Lungs.	Brain.	Eyes.
		Whole blood.	Plasma.	Whole blood.	Plasma.											
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
297†	¾ of pancreas was removed 4 months ago. Did well up to 1 month before experiment, on bread and soup. Then began to lose weight and look sick. ½ hr. before death 0.75 gm. of dextrose per kilo intravenously. Urine contained no sugar at beginning.	0.14	0.18	0.37	0.68	0.65	0.13	0.33	0.77	0.73	0.25	0.22	0.24	0.32	0.05	
D_1‡	Total pancreatectomy 3 days before; D:N ratio before experiment 2.00. Intravenous sugar 4 gm. per kilo 1½ hrs. before tissues taken.	0.31	0.40	1.15	1.43	0.95 (0.95)	0.25 (0.47)	0.45 (0.57)			0.56 (0.53)					0.52

* All values in parentheses are obtained after boiling with 1.8 per cent hydrochloric acid for 1½ hours with a reflux condenser.

** Additional tissues: pancreas remnant 0.08 per cent; testes 0.08 per cent; thyroid 0.03 per cent; trachea 0.11 per cent; esophagus 0.07 per cent; aorta 0.14 per cent; submaxillary gland 0.06 per cent; costal cartilage 0.02 per cent.

*** Additional tissues: testicle 0.25 per cent; thyroid 0.20 per cent; salivary gland 0.37 per cent; bladder 0.16 per cent. Bladder urine at death contained no sugar.

† Additional tissues: pancreas remnant 0.26 per cent; thyroid, trace; esophagus 0.25 per cent; trachea 0.26 per cent; uterus 0.41 per cent; submaxillary gland 0.11 per cent; bladder 0.29 per cent; aorta 0.38 per cent.

‡ Muscle taken before sugar was given showed 0.17 per cent, after hydrolysis 0.18 per cent. Only a trace of glycogen was present. Muscle after sugar injection contained 0.08 per cent glycogen and liver 0.06 per cent. Plasma sugar directly after injection of sugar was 1.78 per cent.

TABLE X.

Comparison between Normal and Diabetic Animals. Liver and Muscle Tissues.

Plasma dextrose.		Liver dextrose.		Muscle dextrose.		Heart muscle dextrose.	
Normal.	Diabetic.	Normal.	Diabetic.	Normal.	Diabetic.	Normal.	Diabetic.
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0.21	0.23	0.80	0.59	0.06	0.09	0.05	0.06
0.24		0.37		0.11		0.12	
0.25				0.09		0.29	
0.25		0.56		0.27		0.31	
0.26	0.26	0.23	0.26	0.13	0.06	0.21	0.04
0.27	0.27	0.32	0.83	0.10	0.05	0.22	0.15
0.29	0.29	0.51	0.40	0.14	0.07	0.23	0.14
0.32	0.29	0.29	0.22	0.25	0.05	0.21	0.11
0.40	0.38	0.62	0.59	0.10	0.21	0.08	
0.41	0.44	0.55	0.20	0.25	0.02	0.22	0.08
	0.53		0.34		0.05		0.18
	0.56		0.37		0.26		0.28
	0.58		0.49		0.12		0.13
0.63	0.68	0.47	0.65	0.21	0.13	0.08	0.33
0.83	0.71	0.75	0.35	0.33	0.14	0.33	0.10
1.05	1.00	0.75	0.48	0.41	0.33	0.56	0.27
	1.43		0.95		0.25		0.45

Increase in the Reducing Power of the Tissue Extracts after Boiling with Hydrochloric Acid.

In many of the experiments the tissue extracts were hydrolyzed to determine the amount of sugar present as polysaccharides. The results are of sufficient interest to make it desirable to arrange these data separately in Table XI.

It is noted that occasionally, but not always, there is an appreciable increase in the reducing power of the muscle extracts after hydrolysis. This applies both to skeletal and heart muscles, although the percentage increase in the thigh muscle is often greater than in the heart. In all the normal dogs, only three, Nos. 355, 361, and P III, failed to show an appreciable increase in the dextrose values on boiling with hydrochloric acid. The reducing power of the muscle extract in Dog P II is nearly trebled on hydrolysis. The diabetic dogs are equally divided, Dogs 322

present. Muscle after sugar injection contained 0.08 per cent glycogen and liver 0.06 per cent. Plasma sugar directly after injection of sugar was 1.78 per cent.

TABLE XI.

Increase in the Reducing Power of the Tissue Extracts after Hydrolysis.

No. of experiment.	Plasma sugar.	Liver.		Skeletal muscle.		Heart.		Stomach.	
		Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.	Before hydrolysis.	After hydrolysis.
				Normal.					
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
N IV	0.11	0.26	0.27	0.07	0.10	0.20	0.25	0.04	0.04
350	0.24	0.37	0.37	0.11	0.20				
347	0.19	0.86	0.85	0.11	0.16	0.13	0.17		
I II	0.25	0.56	0.58	0.27	0.35	0.31	0.35	0.13	0.16
355	0.83	0.75	0.72	0.33	0.32	0.33	0.36	0.46	0.46
361	0.32	0.29	0.27	0.25	0.24	0.21	0.20	0.11	0.12
A II	0.36	0.52	0.52	0.21	0.36	0.23	0.25	0.14	0.17
A III	0.29	0.51	0.56	0.14	0.28	0.23	0.27	0.15	0.17
353	0.18			0.08	0.12				
P II	0.26	0.23	0.27	0.13	0.32	0.21	0.29	0.15	0.19
P III	0.47	0.55	0.57	0.25	0.29	0.26	0.27	0.22	0.27
				Diabetic.					
322	0.56	0.26	0.26	0.28	0.29			0.29	0.29
397	0.38	0.59	0.59	0.21	0.30				
271	1.00	0.48	0.48	0.33	0.32	0.27	0.29		
D I	1.43	0.95	0.95	0.25	0.47	0.45	0.57	0.56	0.53

and 271 show no increase, No. 397 shows a slight increase, and No. D I nearly doubles the dextrose on hydrolysis. Apparently the presence or absence of hydrolyzable sugar in the muscles does not depend on the presence or absence of diabetes. Although the administration of sugar both in normal and diabetic animals increases the amount of dextrose found after hydrolysis, when this amount is compared with the sugar present as dextrose the percentage increase is about the same with or without sugar administration.

Examination of Table XI reveals further that the striated muscle of dogs is the only tissue which contains any appreciable amount of sugar in the polysaccharide state. Extracts of stomach and liver, as well as kidney, lung, and skin, never show a change in reducing power on hydrolysis that is not well within the limits of error in experimental technique.

Amount of Dextrose Recovered after Injection.

In several of our experiments the data at hand were utilized to estimate what proportion of dextrose injected into the animal could be accounted for (Table XII).

TABLE XII.

Amount of Dextrose Recovered after Injection.

No. of experiment.	Weight of dog.	Route of sugar administration.	Sugar given.	Sugar recovered.		Time after sugar given before analysis.	Remarks.
	kg.		*gm.*	*gm.*	*per cent*	*hrs.*	
350*	15.0	By mouth.	97	94.4	97	1	
P III	15.5	Intravenous.	62	57.2	92	1½	
347**	16.0	By mouth.	192	159.0	83	2	
D I	18.7	Intravenous.	76	53.2	70	1½	
297	16.3	"	24	15.4	64	½	Partial pancreatectomy but not glycosuric.
I_2	14.5	"	52	31.9	61	1½	
P II	11.0	"	40	21.8	55	1½	

* Experiment was performed under conditions similar to those in Lusk's metabolism work (19).

** Levulose was the sugar used in this experiment.

As it was not possible to estimate the respiratory metabolism, Dog 350 was given sugar under conditions similar to those of Lusk (19) when the amount of sugar burned was followed for several hours after its introduction into the stomach. A 15 kilo dog was given 97 gm. of dextrose in 170 cc. of water by stomach tube and killed an hour later. In estimating the sugar in the tissues at the beginning of the experiments the average of the normal values in Table I was used. In computing the amount of sugar recovered the volume of blood was estimated as amounting to one-twelfth of the body weight (20); the muscles as three-sevenths of the body weight. Details of the calculation are given below. The weight of the dog was 15 kilos.

	gm.	
1. Amount recovered from stomach contents	77.5	
2. " " " " itself	5.1	
3. " " " intestines	0.9	
4. " " " blood. 1250 × 0.10 per cent	1.3	
5. Amount recovered in muscles. 6429 × 0.05 per cent	3.2	
6. Amount recovered in liver, kidneys, lungs, and skin	1.9	
7. Amount burned computed on basis of Lusk's experiment	4.5	
Total recovered	94.4	
Amount given	97.0	
Amount unaccounted for	2.6	
Recovered	97	per cent.

This method for estimating the recovered sugar is used in the other animals reported except that no estimation of amounts that may have been burned was possible. Following levulose by mouth and dextrose intravenously the amounts of sugar burned are unknown. Glycogen estimations were not made in all cases. Dogs D I and P II showed no glycogen, while in Dog P III there was 0.16 per cent in the liver and 0.34 per cent in the muscles. Variable amounts of sugar were accounted for. In Experiment 350, where it was possible to estimate the dextrose burned, 97 per cent was recovered. In Dog P III after intravenous dextrose we were able to account for 92 per cent. The amount of dextrose burned in each case is important in attempting to account for the disappearance of dextrose after injection, and especially so when sugar is given intravenously, because Lusk has shown that the higher the concentration of metabolisable material in the blood stream, the higher the metabolism.

Only one opportunity to examine human tissue presented itself during this investigation. A boy 9 years old died in diabetic coma, 2 years after onset. 24 hours before death his blood sugar was 0.50 per cent, and his liver, obtained 1 hour after death, contained 0.55 per cent of dextrose; no change after hydrolysis. There was no glycogen in the liver.

DISCUSSION.

From the data presented it is clear that under widely varying conditions the amount of carbohydrate existing as dextrose in the tissues at any one time is small. The tissue of most importance is muscle, for it comprises three-sevenths of the body weight and by its mass could be responsible for storing large amounts of free dextrose. Four normal animals, having an average blood sugar of 0.11 per cent, had an average of 0.08 per cent in the skeletal and heart muscle. These values are much lower than the 0.2 to 0.4 per cent found by Pavy, who does not give any blood sugar figures. Although sugar administration increases the amounts of sugar to be found in the tissues, its concentration is always low and not commensurate with the increase in blood sugar. The highest value, 0.41 per cent, for muscle sugar was found after intravenous injection of 5.5 gm. per kilo, in which the plasma sugar rose to 1.05 per cent. Except in the phlorhizinized dog, No. 353, with a plasma sugar of 0.08 per cent and a muscle sugar of 0.08 per cent, we have never found dextrose in the muscle to equal that in the blood. Frequently the muscle sugar was only half as concentrated as the blood sugar, and in some instances in normal animals, as Dog 337, amounted to only one-fourth.

Diabetic animals present little difference in respect to dextrose in muscles. The amounts found are small, and apparently not influenced by the type of diabetes or sugar administration. As in the normal animals, the controlling factor seems to be the concentration of dextrose in the blood. The point of difference between the normal and diabetic animal is that when comparative blood sugar values are taken the muscle of the diabetic animals contains less dextrose than the normal. This fact suggests that diabetic tissues are more impermeable to sugar than normal ones, and coincides with the results of previous workers, that sugar injected into the veins of diabetic animals does not leave the blood so rapidly as it does in normal animals. Just what relation this phenomenon may bear to glycogenesis and the oxidation of glucose in the organism is unknown and difficult to conjecture. As all the tissues contained more or less blood, the actual amount of dextrose in the muscle substance must have been very small indeed. It is reasonable to expect that the amounts of blood remaining in the

tissues in animals killed under similar conditions were fairly constant. Therefore the difference between normal and diabetic animals gives support to the belief that, contrary to the findings of Albertoni and Monetti, dextrose does exist in the muscles, at least in normal animals.

That body tissues ordinarily contain only small amounts of free dextrose has an important bearing on all discussion of the fate and probable disposition of dextrose in the organism. Body fluids and tissues have been credited with the same concentration of dextrose as exists in the blood. In amount at least, this allowance is far too liberal. We have no way of telling now nearly the animal experiments correspond to what takes place in normal and diabetic men. But granting that the fate of glucose is somewhat similar, it is no longer possible to explain the retention of carbohydrate by diabetics as in the form of dextrose distributed throughout the tissue and body fluids. In the case of Gerald S. reported by Allen and Du Bois (21), the non-protein respiratory metabolism showed over a period of 2 weeks 438 calories derived from carbohydrates, while the carbohydrates in the food were only 2 gm. daily. As the individual weighed 40 kilos, the muscle tissue computed at three-sevenths of the body weight amounts to 17.7 kilos. Allowing a diminution of 0.10 per cent in the concentration of dextrose in the muscles during this time as contributing to the carbohydrate calories, only 17.7 gm. are available. An allowance of 0.10 per cent reduction in the muscles, judging from the results of animal experimentation, is liberal when one considers that Gerald S.'s blood sugar varied between 0.156 per cent and 0.208 per cent during the period of observation in question. As Allen and Du Bois and others have shown that acetone bodies cannot exist in quantities sufficient to account for more than a small part of the increase in the respiratory quotient of these cases, the evidence points strongly to glycogen as furnishing the extra calories.

It is necessary in this connection to consider the accuracy with which it is possible to estimate the amount of carbohydrate burned over long periods when computing the amount of sugar retained by the diabetic individual. Under the usual conditions there is a possible error of 6 to 10 per cent in the determination of the respiratory quotient because the second figure in the quotient is

accurate to within 2 or 3 only, although under ideal circumstances an error of only 1 in the second place may be secured. Hence a man could burn 50 gm. of dextrose at the rate of 2 gm. per hour in 24 hours, impossible of detection by the calorimeter. On the other hand, an organism may be accredited with the combustion of more sugar than actually occurs, at least within these limits. In diabetes the situation is further complicated when acidosis is present.

With the exception of the liver, which holds a particular place in carbohydrate metabolism, the several tissues examined besides muscle reveal little that we consider of importance. No evidence has come to light that any of the tissues have unusual power to store dextrose in high concentration. In general it may be said of the minor tissues that the concentration of dextrose varies more or less with vascularity. The brain, which contains very little blood, contains exceptionally low amounts of dextrose. In Dog 352 after intravenous dextrose only 0.11 per cent was found in the brain. The average under the varying conditions is considerably below this figure.

Analyses were made of the stomach for the purpose of comparing smooth with striated muscle. In nearly every instance the concentration in the stomach musculature approximated that found in the striated muscles.

The determination of dextrose in the liver involves several difficulties not easy to overcome. From examination of Table III it is evident that the manner in which the animal is killed, as well as the rapidity with which enzyme action is checked, is most important. But although these factors were controlled as well as possible, there exists a wide variation in the amount of dextrose found. This variation seems most probably due to difference in glycogenolysis in the several animals. In the normal animals, P II and P III, known to be glycogen-free, and in the diabetic dogs, the liver dextrose was relatively low. Only two of the diabetic dogs, Nos. 300 and 292, have concentrations of dextrose in the liver higher than that in the plasma. In Experiment 300, owing to an accident, the liver was not thrust into boiling water for 3 or 4 minutes after excision. Dog 292 was killed early in the work by bleeding, before the effect of bleeding on glycogenolysis in the liver was appreciated. In the light of Bang's experi-

ments the higher values in the livers of normal animals might be interpreted as meaning an ability, not possessed by the diabetic dogs, of storing dextrose in higher concentration than it existed in the blood. By taking especial precautions to prevent glycogenolysis we were unable to confirm Bang's results, nor are we inclined to attribute the difference in values between normal and diabetic animals as having any such significance. The marked rapidity with which glycogenolysis occurs in the liver, as compared to the muscles, has been observed by Panormoff (22) and others.

By hydrolysis with hydrochloric acid the extracts of heart and skeletal muscle showed an increase in their power to reduce copper. No attempt was made to determine the nature of the substance yielding extra sugar, but previous work suggests that it may be due largely to a biose, probably maltose.

By treating the extract of dog muscle with phenylhydrazine hydrochloride Panormoff (22) obtained a crystalline substance with a low melting point which he was unable to identify. An osazone obtained from the liver isolated by Röhmann (23) was considered by them to be isomaltose, but no details are reported. Pavy and Siau (24) by first precipitating the proteins of the blood with alcohol and extracting the coagulum with water, obtained an extract which yielded a copper-reducing substance after boiling with hydrochloric acid. An osazone was obtained melting at 153° and considered by them to be isomaltosazone because of its resemblance to Fischer's substance which he called "isomaltose." A similar osazone was isolated by Osborne and Zobel (25) melting at 153°, but on further purification they were able to raise the melting point from 153° to 162°. Hence they concluded that the substance was not isomaltosazone, but in reality maltosazone contaminated by dextrinous bodies. Furthermore, these workers showed that when muscles were washed free of blood by salt solution the muscle juices hydrolyzed glycogen into a substance which they considered to be maltose. The results of Levene and Meyer (26) are interesting in this connection. They found that if muscle juice (rabbits) and pancreas extract were allowed to act on a solution of dextrose a condensation occurred. A biosazone was isolated with a melting point of 200°.

The evidence at hand strongly favors the presence in muscle of higher sugars or polysaccharides, of which maltose probably constitutes the larger part. The fact that no hydrolyzable sugar exists in other tissues than muscle is interesting, but permits of no special interpretation, particularly because it occurs in both normal and diabetic animals.

Attempts to account for all the dextrose injected into the ani-

mal, similar to the experiences of other investigators, have not been wholly satisfactory. The difficulties attending such an investigation have been discussed by Joslin (27). After injection of dextrose the amount excreted unutilized in the urine and the quantity burned may be determined with some accuracy, provided a calorimeter is available. The amount retained as dextrose in the blood and tissues is much more difficult to estimate, as is also the amount transformed into glycogen, polysaccharides, and fat. Little is known about the incompletely oxidized products of sugar. Attention should be called to the frequently neglected changes in blood volume following the intravenous injection of large amounts of dextrose. A calorimeter was not available in our work; hence our observations cannot be considered complete. To overcome this difficulty partially Dog 350 was given dextrose under conditions similar to those used by Lusk in determining the amount and rate at which dextrose was oxidized after mouth administration. We were able to account for 97 per cent of the administered dextrose in this dog. Where levulose, 12 gm. per kilo, was introduced by stomach tube in Dog 347, only 83 per cent was accounted for, but this did not include any estimate of the amount burned. Johansson (28) found that the CO_2 output after levulose administration in animals and men was frequently double what it was after the injection of dextrose. Considerable levulose may have been oxidized in the 2 hours elapsing between its injection and the killing of the animal. After the intravenous administration of 4 gm. per kilo and killing in $1\frac{1}{2}$ hours, 92 per cent of the amount given could be accounted for, while in others less amounts were recovered. During the short period between sugar injection and the killing of the animal it is usually taken for granted that little glycogen is formed. Freund and Popper's (29) work confirms this view. But that glycogen may be formed in a short period of time is shown in Dog P III, which was made glycogen-free by phlorhizin and adrenalin (the muscles proved to be free of glycogen by analysis before sugar was given), and which had an appreciable amount of glycogen, 0.34 per cent, in the muscles $1\frac{1}{2}$ hours after dextrose administration. It is possible, however, that glycogen was unevenly distributed in the muscles and the sample taken at the beginning of the experiment did not have any, but the remaining muscle still contained small amounts.

The conditions governing the formation of glycogen are not well known. Johansson observed, in men who had their glycogen reserve depleted by starvation or severe muscular work, that the CO_2 output after sugar administration was much less than in men with a normal amount of glycogen. He logically interpreted this fact as indicating a much more rapid formation of glycogen following ingestion of sugar by glycogen-poor organisms than under normal conditions. Other not well known factors in glycogen formation doubtless play a part. When all the difficulties encountered in attempting to trace the fate of ingested dextrose are considered, it is not strange that wide variations in results are found, also that there is more or less sugar for which no account can be given.

SUMMARY.

1. The concentration of dextrose has been determined in muscle, liver, heart, kidney, spleen, pancreas, stomach, intestine, skin, lung, and eye tissues of normal and diabetic animals, under varying conditions. In a few instances the dextrose has been estimated in the bladder, parotid glands, esophagus, uterus, tongue, diaphragm, thyroid, aorta, trachea, and brain.

2. No difference in the amount of dextrose to be found in either normal or diabetic tissues is caused by variation in the manner of producing a hyperglycemia; that is, administration by mouth, subcutaneously, intravenously, or intraperitoneally.

3. The concentration of dextrose in the tissues varies directly with the degree of hyperglycemia. The largest amount of sugar was found in the liver, the smallest quantity in the brain, the difference being largely accounted for by difference in vascularity.

4. The concentration of dextrose in the tissues is invariably lower than the blood sugar except in the liver, where the higher concentrations found may be explained by rapid glycogenolysis. In the muscles the amount of dextrose is low, varying in normal animals between 0.04 per cent when the blood sugar is 0.10 per cent and 0.41 per cent when the blood sugar reaches 1.05 per cent after intravenous sugar; while in diabetic animals the muscle sugar falls within the normal limits even though the hyperglycemia rises as high as 1.43 per cent.

5. Normal animals have a higher concentration of dextrose in

striated muscle than diabetic animals when the levels of blood sugar are comparable.

6. Bleeding of the animal causes a rapid glycogenolysis in the liver.

7. Adrenalin produces no change in tissue sugars different from that found when hyperglycemia is caused by other means.

8. In the striated muscles of many but not all normal and diabetic animals there is an increase in the reducing power of the extract after boiling with hydrochloric acid. No such changes could be demonstrated in the extracts of other tissues. The polysaccharide mother substance of the sugar formed by hydrolysis is not glycogen, and may be present when glycogen is not.

9. Amounts of dextrose varying between 55 and 97 per cent of the amount injected were accounted for. The maximum of 97 per cent was recovered in an animal where it was possible to calculate the amount of sugar burned.

10. The liver of a boy who died in diabetic coma, obtained 1 hour after death, contained 0.55 per cent of dextrose and no glycogen. His blood sugar 24 hours before death was 0.50 per cent.

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THE EFFECT OF HIGH TEMPERATURES ON THE NUTRITIVE VALUE OF FOODS.

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(Received for publication, March 17, 1917.)

In an earlier publication[1] from this laboratory results were cited which indicated that young rats are unable to grow on a diet that has been subjected to high temperatures. Many investigators believe that the so called accessories are destroyed by exposure to heat, and as that is a point of great importance, further studies were made on the nutritive properties of heated foods.

In these investigations white rats were used as experimental animals. Many of them were at least half grown when the feeding trials began, but some were quite immature. In some cases corn constituted the ration, in others a mixture of corn and egg white, one or both of which had been heated in an autoclave. In every case a salt mixture was added to the diet to compensate for the mineral deficiencies of maize. The heating process was continued for 6 hours at 30 pounds' pressure.

It soon became evident that heating the egg white had little or no effect; but in every case in which the corn was heated, the diet was inadequate in some respect, and the animals were unable to maintain their body weights. These facts are presented in greater detail in Charts 1 to 4.

It has been suggested, however, that some factor other than that of accessories was altered. If such were the case probability seemed to indicate that the protein of the ration had been changed in some way. In order to investigate that point, a comparatively pure protein, egg white, was heated in an autoclave for 6 hours at 30 pounds' pressure. This was carefully dried, and combined

[1] **Hogan**, A. G., *J. Biol. Chem.*, 1916, xxvii, 193.

with protein-free milk, butter, starch, and agar, in such proportions that the protein formed approximately 9 per cent of the ration. This diet did not permit maximum growth and possibly these rats grew somewhat more slowly than other animals receiving a similar ration in which the protein had not been heated. Evidently, however, the nutritive value of the protein had not been seriously impaired.

A series of feeding trials similar to the one just described was conducted with casein in the ration instead of egg white. In these experiments the casein was heated in an autoclave for 2 hours, but at different pressures. In one case the protein was heated at 15 pounds', in another at 30 pounds', and in a third at 45 pounds' pressure. A control lot received unheated casein. In this series the protein formed approximately 12 per cent of the ration. In all cases animals of the same sex grew at approximately the same rate, indicating that the nutritive value of the casein had not been materially lowered by the high temperature.

The data are presented graphically in Charts 5 to 9.

SUMMARY.

The heating process described in this paper does not materially lower the nutritional value of protein.

It is suggested that one or more of the so called food "accessories" may be injured by high temperatures.

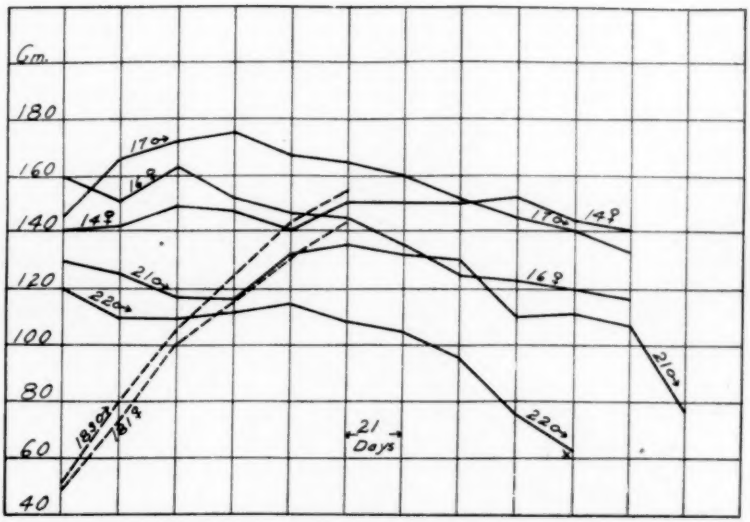


CHART 1. The animals shown in this chart received as their sole diet corn that had been heated in an autoclave. All of them slowly lost weight, and Rat 22' died. To show that rats are able to grow on corn that has not been heated, some additional curves are reproduced here. The broken lines representing Rats 181 and 183 are typical of a fairly large number that have grown practically to maturity in this laboratory on corn alone (that had not been heated). *x* at the end of a curve indicates the animal's death.

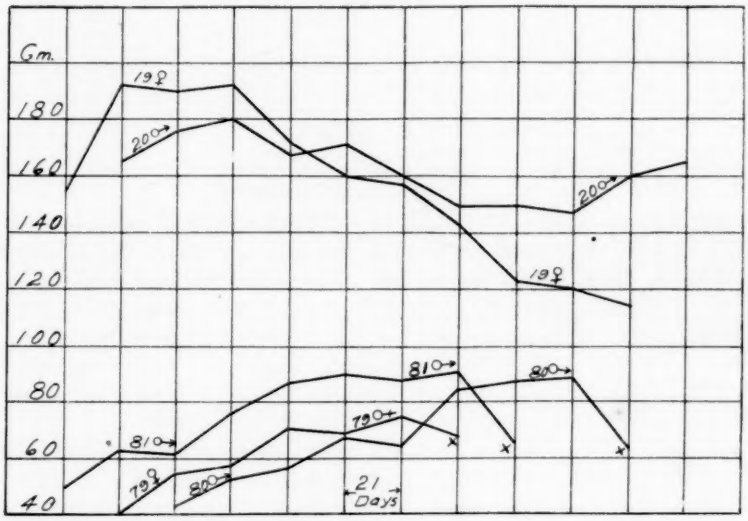


CHART 2. The animals shown in this chart received a diet similar to the one fed to the rats in Chart 1. This ration contained a much larger percentage of protein, however. Enough of the autoclaved egg white was added to make the protein content of the diet approximately 20 per cent. The younger animals failed to grow and finally died. During the experimental period the older animals steadily lost weight. *x* at the end of a curve indicates the animal's death.

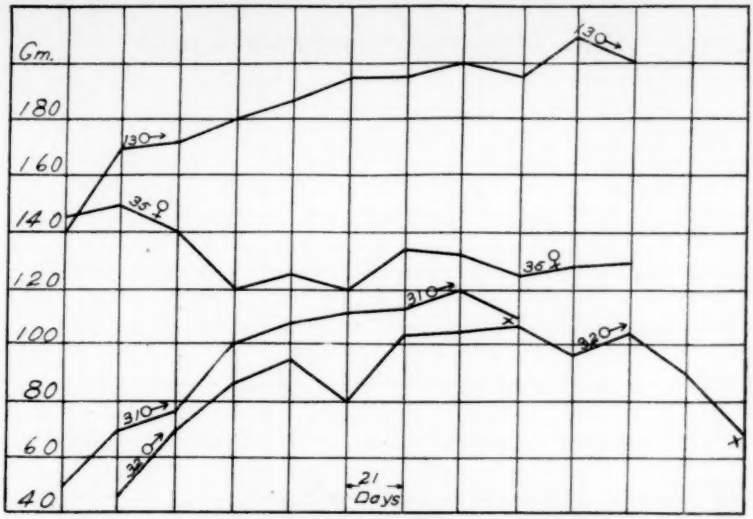


CHART 3. These animals received corn that had been subjected to high temperatures. In addition they received unheated egg white, in amounts comparable to that given to the rats shown in Chart 2. The two younger animals grew slowly for a time, but finally died. Of the two older rats, the male made some growth, but the female gradually lost weight. x at the end of a curve indicates the animal's death.

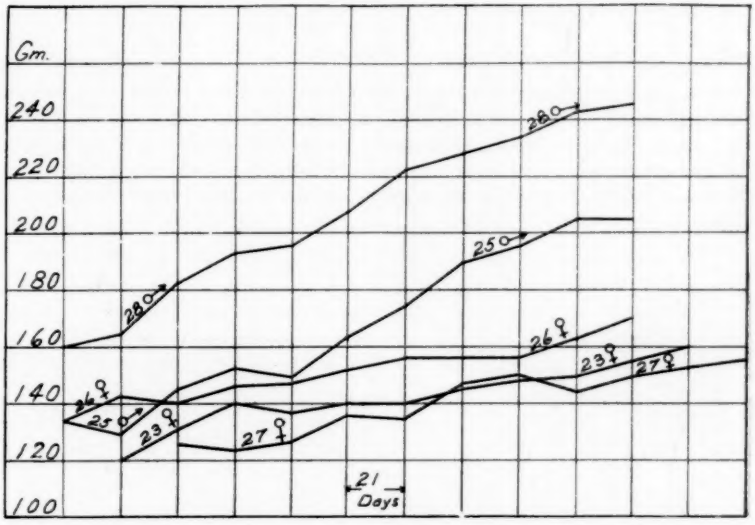


CHART 4. The diet received by these animals consisted of unheated corn and autoclaved egg white. All animals grew slowly, but at the usual rate for this ration.

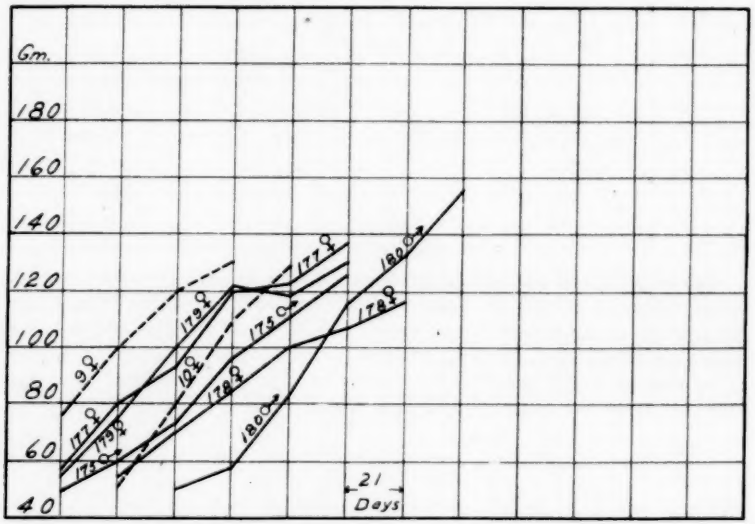


CHART 5. The protein constituted 9 per cent of the ration in this case, and consisted of egg white instead of casein. The egg white had been heated in the autoclave for 6 hours at 30 pounds' pressure. The broken lines represent the growth of rats on a similar diet, in which the egg white had not been heated. The composition of the ration was as follows.

	gm.
Egg white	100
Protein-free milk	257
Butter	300
Starch	325
Agar	20

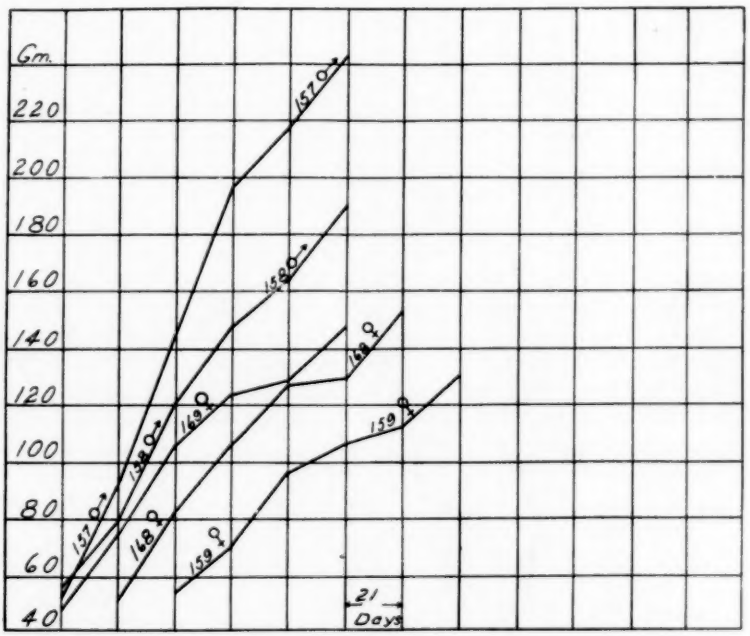


CHART 6. The diet fed to the animals shown above included approximately 12 per cent of unheated casein. These rats serve as controls for Charts 7, 8, and 9. Of the males, Rat 157, and of the females, Rat 169 grew most rapidly, and for convenience their growth curves are inserted in the following charts. The ration is as follows.

	gm.
Casein	152
Protein-free milk	257
Butter	300
Starch	295
Agar	20

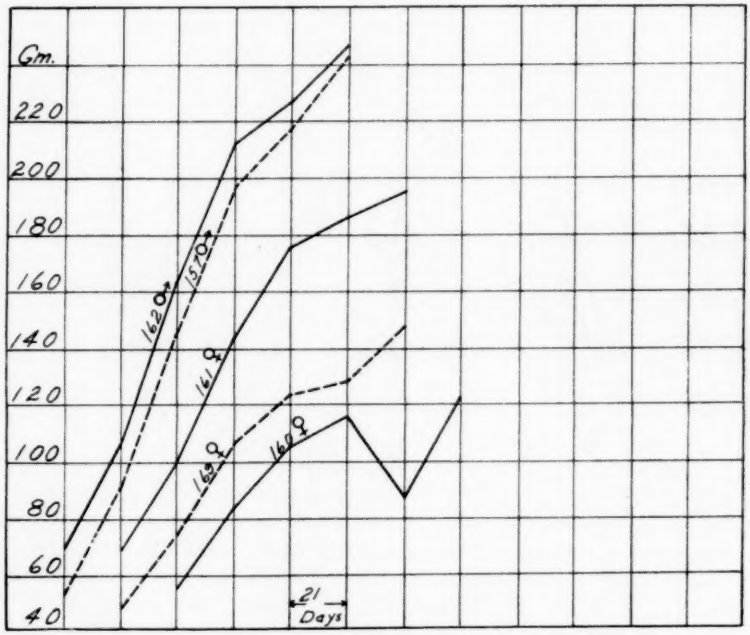


CHART 7. The protein in the ration of these animals had been heated in an autoclave for 2 hours at 15 pounds' pressure. The broken lines show the growth of control animals, taken from Chart 6.

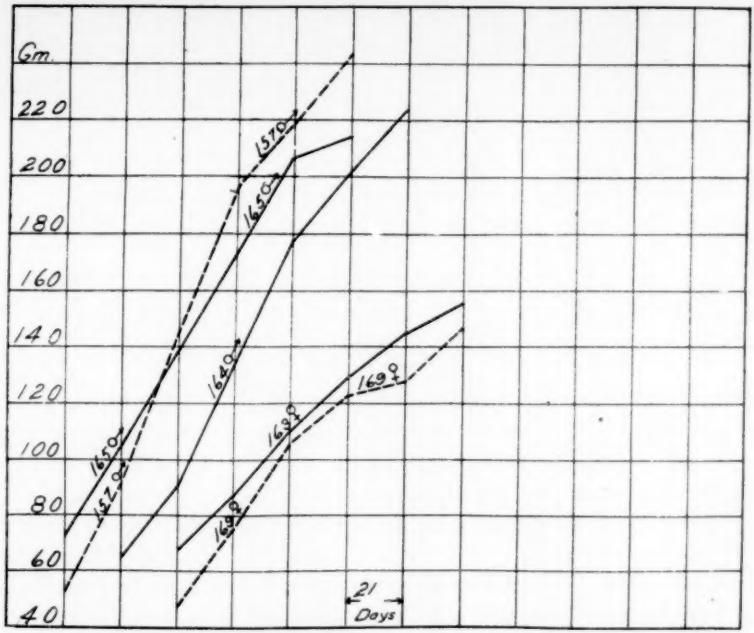


CHART 8. The casein used in preparing the diets of these animals had been heated at 30 pounds' pressure for 2 hours. Evidently the nutritional value of the casein had not been impaired.

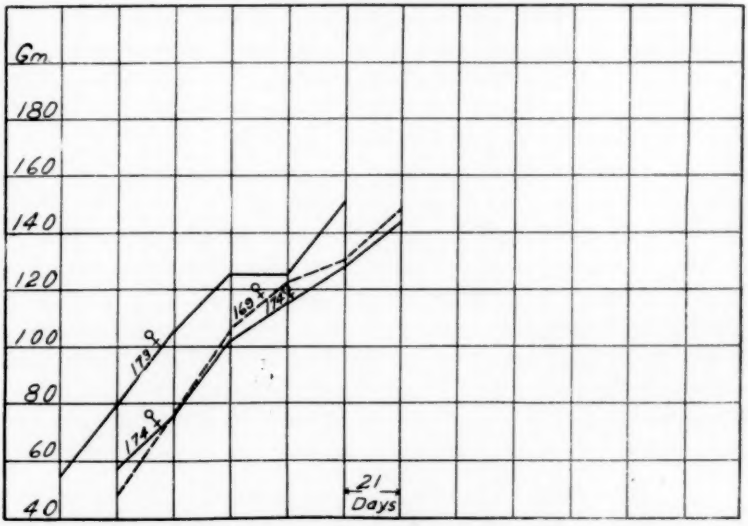


CHART 9. These animals received casein that had been heated in the autoclave for 2 hours at 45 pounds' pressure. As both of these animals were females, the curve of Rat 157, Chart 6, is omitted.

A QUANTITATIVE TEST FOR SMALL AMOUNTS OF SUGAR IN THE URINE.

By ALMA HILLER.

(From the Medical Clinic of the Johns Hopkins Hospital, Baltimore.)

(Received for publication, March 6, 1917.)

A rapid, accurate method for the determination of small amounts of sugar in the urine is not available at present. To overcome this uncertainty, a micro method was adopted. By adapting the blood sugar method of Lewis and Benedict,[1] even traces of urinary sugar may be determined quantitatively with great precision.

Since the colorimetric estimation of Lewis and Benedict depends upon the reaction of the sugar with alkali and picric acid, and a similar color reaction is obtained by the action of alkali and picric acid on the creatinine and other substances normally present in urine, these must be removed before the method can be applied. Normal urines without sugar yield a color reaction with the Lewis and Benedict method equivalent to 0.05 to 0.1 per cent glucose. In order to remove these interfering substances, the process recently suggested by Folin[2] was used.

The complete method is as follows:

To about 10 cc. of urine are added 2 gm. of picric acid and 2 gm. of bone-black, the mixture is shaken for about 5 minutes and filtered.[2] 2 cc. of this filtrate are used for the determination, and placed in a 25 cc. volumetric flask. Then 15 cc. of saturated aqueous solution of picric acid are added and the flask is filled to the mark with distilled water and shaken. Filtration at this point is not necessary. 8 cc. aliquots are measured into large Jena test-tubes for duplicate determinations. 2 cc. of saturated picric acid solution and exactly 1 cc. of 10 per cent sodium carbonate are added, then one drop of mineral oil, and the contents of the flask are evaporated rapidly over a direct flame until pre-

[1] Lewis, R. C., and Benedict, S. R., *J. Biol. Chem.*, 1915, xx, 61.

[2] Folin, O., *J. Biol. Chem.*, 1915, xxii, 327.

cipitation occurs. About 3 cc. of water are added, the tube is again heated to boiling to dissolve the precipitate, the contents of the tube are cooled, transferred quantitatively to a 10 cc. volumetric flask, made up to mark, shaken, and then filtered through cotton into the colorimeter chamber. The color is compared with a picramic acid standard:

Picramic acid	0.064 gm.
Sodium carbonate (anhydrous)	0.100 "
Water to make	1,000 cc.

Calculation:

$$\text{Per cent of sugar} = \frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.1$$

Tables I and II indicate that creatinine and other substances which give a color reaction with alkaline picrate are entirely removed by the picric acid and bone-black process.

TABLE I.

The Method Applied to Glucose Added to Urine.

Specimen.	Substance added.	Preliminary treatment.	Result, glucose.
			per cent
Urine.	None.	None.	0.084
"	0.1 per cent glucose.	"	0.183
"	0.1 " " "	Bone-black.	0.024
"	0.1 " " "	" + picric acid.	0.100
"	0.1 " " "	None.	0.128
"	0.1 " " "	Bone-black.	Not readable.
"	0.1 " " "	" + picric acid.	0.100

TABLE II.

The Method Applied to a Pentose.

Specimen.	Substance added.	Preliminary treatment.	Result, glucose.
			per cent
Urine.	None.	None.	0.054
"	0.1 per cent *l*-xylose.	"	0.153
"	0.1 " " "	Bone-black.	0.070
"	0.1 " " "	Picric acid.	0.153
"	0.1 " " "	Bone-black + picric acid.	0.101

As the tables show, this procedure is applicable to urines containing pentoses as well as glucose. The method proved to be valuable in estimating the quantity of sugar excreted in a case of pentosuria, in which the concentration of this sugar was only 0.1 to 0.3 per cent.

After the completion of the above article the writer's attention was called to a method published by V. C. Myers,[3] which is similar to, but not identical with her own.

[3] Myers, V. C., *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 178.

THE IDENTIFICATION OF THE PENTOSE IN A CASE OF PENTOSURIA.

By ALMA HILLER.

(From the Medical Clinic of the Johns Hopkins Hospital, Baltimore.)

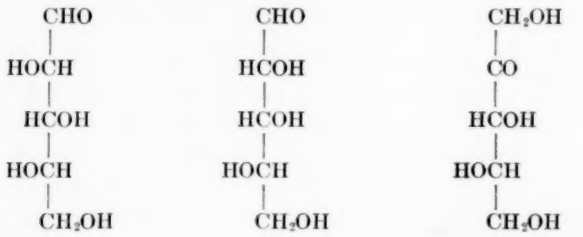
(Received for publication, March 6, 1917.)

Attempts to analyze the pentose eliminated in cases of pentosuria have led to a variety of conclusions. Since the first proof of the excretion of a pentose by Salkowski in 1892 (1), the majority of the earlier cases were identified as inactive *d,l*-arabinose, chiefly by Neuberg (2). Instances of active, dextrorotatory pentose were demonstrated by Blumenthal (3), Rosenfeld (4), and Schüler (5). Active levorotatory pentose was found by Adler (6) and Blum (7). Elliott and Raper (8) attempted to identify their pentose as *d,l*-ribose. Luzzatto (9) and Klercker (10) both demonstrated the dextrorotatory *l*-arabinose. More recently, Levene and La Forge (11) established the identity of their pentose with the osazone of *d*-xylose. Zerner and Waltuch (12) also place their three cases in the *d*-xylose group.

The subject from whom the material for this investigation has been derived was a Russian tailor, aged 28. A diagnosis was made of a low grade of chronic nephritis, moderate hypertension, duodenal ulcer, and pentosuria. The period in which the urines were collected for the determinations reported here began with the first admission of the patient to the hospital in March, 1916, and continued for 8 months, the patient cooperating willingly while in his own home.

The pentose reported in the present observation gave an osazone which, after having been recrystallized from 30 per cent alcohol, melted at 159°C. An osazone formed from *l*-xylose and recrystallized in the same way melted at 161°C. Equal amounts of these osazones were then dissolved in hot 95 per cent alcohol, mixed, and the osazone was crystallized out. These crystals melted at 208°C., and decomposed at 215°C., showing the for-

mation of a mixed *d,l*-xylose, a characteristic reaction of the xylosazones (13), which was used by Zerner and Waltuch (12) and by Levene and La Forge (11) in their identifications. The characteristic mutarotation of xylosazone (Table III) further strengthened this point, and excluded the possibility of ribose or arabinose, and limited the choice to one of three pentoses, whose osazones give these reactions characteristic of *d*-xylosazone. These are *d*-xylose, *l*-lyxose, and *d*-xyloketose, which have the following formulas:



d-Xylose. *l*-Lyxose. *d*-Xyloketose.

By a determination of the direction of rotation of the sugar itself, it would be possible to eliminate one or two of the three pentoses. Since the concentration of pentose in the urine was very slight, varying between 0.1 and 0.3 per cent, the urine had to be concentrated before a satisfactory rotation could be read. The urine without concentration, decolorized by animal charcoal, gave no rotation. This may be accounted for by the fact that through treatment with animal charcoal some of the sugar in solution is adsorbed, especially in dilute solutions, and also to the fact that there are substances normally in urine which give a levorotation. This was shown by Zerner and Waltuch (12) in connection with their work on the specific rotation of the sugar. This apparent inactivity of the decolorized non-concentrated urine might lead to the possibility of mistaking the sugar for an inactive form. For this reason the urine was concentrated according to the method of Zerner and Waltuch with the preliminary precaution, suggested by Bang (14), to prevent loss of sugar by treatment with charcoal. The method in full will be presented in the experimental part. The rotation of a 1.25 per cent solution thus obtained was unmistakably dextro.

This would exclude the possibility of *d*-xylose, which is levorotatory. *l*-Lyxose has been demonstrated by Van Ekenstein and Blanksma (15), and is dextrorotatory. *d,l*-Xyloketose has been demonstrated by Neuberg (16), but *d*-xyloketose is, so far, unknown. But assuming, as Zerner and Waltuch do, that by analogy with *d*-fructose and *d*-sorbose the osazone and the sugar may rotate in the same direction, and that *d*-xyloketose would be dextrorotatory if it were found, the possibility is limited to two sugars, *l*-lyxose and *d*-xyloketose.

Comparing the observed rotations with the calculated ones, no identification with *l*-lyxose can be made. If, however, the levorotation value of normal urines (Zerner and Waltuch) is taken into consideration, approximate values might be obtained which approach the calculated values for *l*-lyxose.

As this evidence is not very convincing, attempts were made to establish the possible identity of this sugar as *d*-xyloketose. This was done by testing for the keto group by oxidation of the concentrated urine with bromine. As the experiments show, there was a very slight loss of the sugar, as compared with the entire loss of the sugar in the aldo control. As a further proof of the presence of the keto group, the test of Pinoff and Gude (17) with ammonium molybdate was applied to the concentrated urine, and the characteristic color reaction was obtained. The resorcinol reaction of Seliwanoff was also positive.

EXPERIMENTAL.

Method for Concentrating the Urine.—To about 500 cc. of urine, freed from toluene by filtration, enough alcohol was added to make a 20 per cent alcoholic solution. This was done in order to prevent the disappearance of sugar on decolorization with charcoal. According to Bang's statement (14) and the present observations, little sugar is lost if these precautions are taken. According to the original method of Zerner and Waltuch, as much as 20 per cent of the sugar could not be recovered, whereas with Bang's method there was a loss of only about 3 per cent. The treatment with blood charcoal and subsequent procedure was identical with that of Zerner and Waltuch.

TABLE I.

Rotation Values of the Concentrated Urine in a 2 Dm. Tube.

Reduction values.	Rotation observed.	Rotation calculated for *l*-lyxose.
per cent		
1.79	+0.24°	+0.49°
1.25	+0.21°	+0.34°

TABLE II.

Rotation of Urine Treated with d-Lyxose and Concentrated According to Zerner and Waltuch.

Reduction value.	Rotation observed.	Rotation calculated
per cent		
1.80	−0.70°	−0.50°

TABLE III.

Mutarotation of the Osazone, 0.025 Gm. in 1.25 Cc. of Pyridine Alcohol, Rotated in a 0.5 Dm. Tube.

Time after preparation of solution.	Rotation.
10 min.	+0.10°
25 "	+0.15°
4 hrs.	+0.24°
7 "	+0.40°

Methods for Identification and Separation of Aldo and Keto Sugars in Solution.

Two procedures were employed, a macro and a micro method, which in detail were as follows.

Macro Method.—The urine was concentrated in the above mentioned manner; in fact the same solution was used after its rotation had been observed. The quantity of sugar present was determined by the Fehling-Benedict method; the solution was then treated with liquid bromine, by adding twice the weight in bromine of the sugar found present. This solution was tightly stoppered and allowed to stand 48 hours, with occasional shaking. At the end of this interval any excess bromine was removed with a separating funnel, and the solution was freed of bromine by

distillation in vacuum at 50° until colorless. The solution was neutralized and another Fehling-Benedict determination made. As Tables IV and V show, in the case of a ketose there is very little loss on reduction. In the case of an aldose, the loss is 100 per cent.

TABLE IV.

Results of Brominization Method.

Solution.	Sugar present.		Loss.	
	Before brominization.	After brominization.		
	gm	*gm.*	*gm.*	*per cent*
Urine concentrated	1.25	1.20	0.05	4
Levulose (ketose)	1.62	1.56	0.06	3.7
Glucose (aldose)	1.38	0.00	1.38	100

Micro Method.—In order to obviate the long process of decolorizing and evaporating under diminished pressure, a method was devised for making the determinations directly on the urine. Since the amounts of the sugar found in the urine were so small (usually 0.1 to 0.3 per cent), the blood sugar method of Lewis and Benedict was modified in order to determine the pentose in the urine quantitatively (18). This having been accomplished, the brominization was carried out as in the macro method.

TABLE V.

Results of Direct Brominization of Urine in the Present Case of Pentosuria.

Sugar present.		Loss.	
Before brominization.	After brominization.		
gm.	*gm.*	*gm.*	*per cent*
0.296	0.256	0.040	13.5

CONCLUSIONS.

The mixed melting point and the mutarotation undoubtedly classify the pentose in the present case of pentosuria in the *d*-xylose group. The rotation of the sugar excludes *d*-xylose; it must therefore be either *l*-lyxose or *d*-xyloketose. The ketose tests point toward the identification of this pentose as *d*-xyloketose.

Acknowledgment is due to Dr. P. A. Levene for testing the purity of the osazones obtained and for suggesting some of the chemical procedures.

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THE DECOMPOSITION OF PROTEIN SUBSTANCES THROUGH THE ACTION OF BACTERIA.

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(From the Chemistry Laboratory of the Oregon Agricultural Experiment Station, Corvallis.)

(Received for publication, February 20, 1917.)

INTRODUCTION.

The function of bacteria in the production of ammonia from the protein substances in soil is well known. How these proteins are acted upon, whether it is a hydrolytic process brought about by enzymatic action, whether an oxidative one, or whether a more complex chemical reaction, is yet an unsettled question. In order to ascertain, if possible, by controlling influencing factors as much as conditions would permit, the chemical changes that proteins undergo when acted upon by bacteria, the cause of partial ammonification of protein substances, and the nature of the reactions in general, the following series of experiments was undertaken.

EXPERIMENTAL.

Series I.

In order to follow out a system by which absolute control of influencing factors might be governed, protein substances as pure as possible were selected for the source of nitrogen and mixed with acid-treated and washed Ottawa sand instead of soil. The following proteins were used: blood fibrin, egg albumin, and peptone. The blood fibrin was the pure substance secured from Eimer and Amend; the peptone was the imported Witte-Rostock brand; the albumin was prepared in this laboratory from the whites of eggs. Instead of using a soil infusion which might contain many varieties of bacteria and protozoa, pure organisms were prepared by the Department of Bacteriology of this Experiment Station

and the sand cultures were inoculated with the same. The following organisms, which are generally recognized as good ammonifiers, were used: *Bacillus subtilis*, *Bacillus mycoides*, and *Bacillus vulgaris*. The optimum moisture content of the Ottawa sand for these bacteria was ascertained so that the greatest possible activity of the bacteria might be obtained.

It has been shown by Bainbridge[1] and corroborated by the recent work of Sperry and Rettger[2] that pure native proteins are not acted upon by bacteria, and the presence of a synthetic solution containing the necessary food elements is necessary. Although all the proteins used in this work are not considered native proteins, in order to control experiments alike, 10 cc. of the following synthetic solution were added to each culture.

	gm.
Mannite	20.0
K_2HPO_4	0.5
$CaCl_2$	0.2
$MgSO_4$	0.2
$FeCl_3$ (10 per cent solution)	0.2 cc.
H_2O to make	1,000.0 "

Each of the experiments was carried out as follows: 50 gm. of the Ottawa sand were introduced into each of several 250 cc. Erlenmeyer flasks and 1 gm. of the blood fibrin, albumin, or peptone was added and thoroughly mixed with the sand. 10 cc. of the synthetic solution were next added, together with enough distilled water to obtain optimum moisture content. The flasks were then sterilized and each was inoculated with one type of the pure cultures, *Bacillus subtilis*, *Bacillus mycoides*, or *Bacillus vulgaris*, placed in the incubator, and allowed to ammonify for 2 weeks at 28–30°C.

During the incubation period some ammonia is volatilized and will be lost unless proper precautions for its retention are provided. In these experiments the collecting of the ammonia volatilized was accomplished as follows: Each of the culture flasks was connected with a flask containing 0.1 N sulfuric acid and to a second flask holding a dilute acid (H_2SO_4) solution. Throughout the incubation period air was slowly drawn, first through the dilute

[1] Bainbridge, F. A., *J. Hyg.*, 1911, xi, 341.

[2] Sperry, J. A., and Rettger, L. F., *J. Biol. Chem.*, 1915, xx, 445.

acid solution, which served to remove the ammonia from the atmosphere and to furnish sufficient moisture to prevent excessive evaporation from the cultures, then through the culture flasks, and finally into the flasks containing the standard acid.

Ammonification was allowed to continue for 2 weeks when incubation was checked and a quantitative determination made of the various forms of nitrogen obtained from the acid hydrolysis of the protein. The determinations made consisted of total nitrogen, amide nitrogen, humin nitrogen, diamino-acid nitrogen, monoamino acid nitrogen, and ammonia. The ammonia that was collected in the standard acid during incubation was determined by titrating the excess acid. That which remained in the culture was determined as follows: To the mixture of sand and protein was added sufficient ammonia-free water to permit the protein to become suspended while the heavier sand settled to the bottom of the flask. The water containing suspended protein could then be decanted off. This operation was repeated until most of the protein was removed from the sand. The small amount which remained was obtained by adding a 15 per cent solution of hydrochloric acid and heating upon the steam bath until protein was sufficiently hydrolyzed and could be washed out of the sand. The ammonia contained in the aqueous solution was then determined by making slightly alkaline with lime water and collecting the ammonia in standard sulfuric acid by means of aeration. After the ammonia had been removed, concentrated hydrochloric acid was added to the water solution until a specific gravity of 1.15 was obtained, and the protein was hydrolyzed by heating under a reflux condenser for 72 hours.

The greater part of the acid was removed from the solution resulting from the hydrolysis by evaporating at boiling temperature to small volume. The solution which remained was transferred to a volumetric flask of 250 cc. capacity, made up to volume with water, and aliquot portions were taken for the determination of the various forms of nitrogen.

The Van Slyke method[3] for the determination of the forms of nitrogen in hydrolyzed protein was used in this work. A few slight modifications were made to adapt the method to the work in hand.

[3] Van Slyke, D. D., *J. Biol. Chem.*, 1911-12, x, 15.

To secure proper data for comparison, the different forms of nitrogen in each of the original proteins were ascertained by taking 1 gm. samples, hydrolyzing, and making the same determinations.

The results of this series of experiments are given in Tables I, II, and III. To afford means of comparison the amounts of the

TABLE I.

Results Obtained with Blood Fibrin.

Organism.	Total N.	Ammonia N.	Amide N.	Humin N.	Diamino-acid N.	Mono-amino-acid N.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Blank	146.9		17.5	4.55	33.6	95.9
Bacillus mycoides	103.5	72.7	17.5	6.65	22.8	58.8
" *subtilis*	92.8	63.3	21.7	9.30	15.0	46.2
" *vulgaris*	149.1	10.8	19.7	11.20	31.8	86.6
Decomposed.						
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Blank	—		—	—	—	—
Bacillus mycoides	29.6		—	31.3+*	32.2	38.7
" *subtilis*	36.8		24.0+	102.1+	55.4	51.9
" *vulgaris*	—		12.6+	143.3+	5.4	9.5

* + indicates an increase in that form of nitrogen.

TABLE II.

Results Obtained with Egg Albumin.

Organism.	Total N.	Ammonia N.	Amide N.	Humin N.	Diamino-acid N	Mono-amino-acid N.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Blank	141.4	—	15.4	3.8	25.9	98.0
Bacillus mycoides	88.9	55.3	15.5	9.4	23.4	46.2
" *subtilis*	90.3	57.5	18.8	3.7	12.9	56.0
" *vulgaris*	123.1	13.3	12.1	3.5	23.3	84.0
Decomposed.						
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Blank	—		—	—	—	—
Bacillus mycoides	37.1		0.6+	143.5+	9.3	57.8
" *subtilis*	36.1		22.0+	5.1	50.0	43.0
" *vulgaris*	12.9		21.4	10.2	10.0	14.3

TABLE III.

Results Obtained with Peptone.

Organism.	Total N.	Ammonia N.	Amide N.	Humin N.	Diamino-acid N.	Mono-amino-acid N.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Blank	153.7	—	15.6	4.4	36.1	98.0
Bacillus mycoides	89.6	78.3	23.2	6.6	22.4	38.0
" *subtilis*	71.4	77.7	28.0	2.6	11.3	30.1
" *vulgaris*	130.6	20.6	19.6	4.3	31.1	75.6

Decomposed.

	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Blank	—		—	—	—	—
Bacillus mycoides	41.8		48.7+	48.9	38.0	61.3
" *subtilis*	46.4		79.5	40.9	68.7	69.3
" *vulgaris*	15.1		25.6+	2.3	13.8	22.9

different forms of nitrogen are given, both in terms of mg. present and percentages of the different forms of nitrogen decomposed.

Inspection of the tables shows clearly that there is a marked difference in the action of each organism upon the proteins used. Furthermore, none of the organisms tried acts to a greater extent upon all the proteins than the others; on the contrary, an organism which ammonifies one protein rapidly may ammonify another protein comparatively slowly. In general, the results indicate that the rapidity and extent to which a protein is ammonified in a certain length of time depend upon the organism used as the acting agent. Thus *Bacillus subtilis* ammonifies the nitrogen of blood fibrin 36.8 per cent and *Bacillus mycoides* 29.6 per cent, while *Bacillus vulgaris* shows but little ammonification during the time allowed for incubation. On the other hand, albumin is ammonified more by *Bacillus mycoides* than by either *Bacillus subtilis* or *vulgaris*. In this protein, however, it is to be noted that *Bacillus vulgaris* ammonified it to the extent of 12.9 per cent. Again, in the case of peptone, *Bacillus subtilis* is the most rapid ammonifying organism, changing 46.4 per cent of its nitrogen into ammonia, *Bacillus mycoides* being second with 41.8 per cent, and *Bacillus vulgaris* least with 15.1 per cent.

The monoamino-acid nitrogen and diamino-acid nitrogen are the forms which appear to be most readily changed. The extent

of transformation varies in the different proteins, depending upon the bacteria used, being greater in the monoamino-acid nitrogen in one case, while in another protein the diamino-acid nitrogen will show a higher percentage.

The humin nitrogen and amide nitrogen constitute only a small percentage of the total nitrogen. Nevertheless, changes worthy of consideration are shown in these fractions. *Bacillus mycoides* causes a consistent increase in the humin nitrogen in all the proteins used, and in the case of blood fibrin all the organisms effected an increase of this form. Work by several investigators indicates that this increase may be due to the presence of a sugar since this substance was employed in the preparation of the synthetic solution. Amide nitrogen also showed an increase in nearly all cases which can be explained only on the basis that there was an actual synthesis of this form.

Series II.

In order to gain a deeper insight relative to the changes that occur during the process of ammonification, a separation of the water-soluble and insoluble portions of the ammonified protein was attempted. This was accomplished by centrifuging the water solution of protein substance after separating from the sand as described in the preceding series. The solid or insoluble material thus obtained was hydrolyzed by boiling with strong hydrochloric acid and one-half the soluble portion was treated in like manner. Determinations of the various forms of nitrogen were made in these hydrolyzed samples, together with the other portion left unhydrolyzed.

The details of the preparation of the cultures were similar to the preceding series. Casein and gliadin were the proteins employed. *Bacillus subtilis* was the only type of bacteria used and a double number of cultures were prepared in order that the period of incubation might be extended over two different lengths of time. Thus, for casein, one culture was allowed to ammonify for 6 days and the other for 8 days, while gliadin, which ammonifies very slowly, was allowed to proceed for 15 and 30 days. The casein and gliadin used were prepared in this laboratory, the former from cow's milk and the latter from the gluten of wheat flour.

TABLE IV.

Results Showing Changes in the Different Forms of Nitrogen during Ammonification of Gliadin by the Organism Bacillus subtilis.

Experiment.		Total N.	Amide N.	Humin N.	Diamino-acid N.	Monoamino-acid N.	Ammonia N.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Blank		100.0	25.92	1.84	4.0	68.6	
After 15 days.	Insoluble matter.	22.24	4.64	1.04	2.40	14.08	51.60
	Soluble "	26.32	9.84	1.65	2.24	13.12	
After 30 days.	Insoluble matter.	11.04	1.84	1.04	0.88	8.88	63.84
	Soluble "	24.24	6.96	4.40	3.38	11.12	
Decomposed.							
15 days		51.44	44.13	47.82+	16.0+	60.37	
30 "		64.72	66.05	195.65+	6.0+	70.86	

TABLE V.

Results Showing Changes in the Different Forms of Nitrogen during Ammonification of Casein by the Organism Bacillus subtilis.

Experiment.		Total N.	Amide N.	Humin N.	Diamino-acid N.	Monoamino-acid N.	Ammonia N.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Blank		100.0	12.71	2.29	17.75	68.60	
After 6 days.	Insoluble matter.	16.13	2.61	2.38	2.53	9.68	45.02
	Soluble "	38.71	15.86	1.69	4.84	17.67	
After 8 days.	Insoluble matter.	16.08	2.68	0.62	3.05	10.18	45.02
	Soluble "	38.05	18.75	1.45	4.72	13.93	
Decomposed.							
6 days		44.56	45.18+	76.66+	58.62	60.26	
8 "		45.86	68.67+	10.00	56.03	64.84	

The results obtained above substantiate, in general, those of the preceding series. All the forms of the nitrogen in the protein used are changed or acted upon by *Bacillus subtilis*. Since there is but a small amount of diamino-acid nitrogen in casein and gliadin, the monoamino-acid nitrogen was evidently the source of most of the nitrogen for the ammonia formed. A comparison of

the rate of ammonification of different proteins shows that they may vary widely, casein and gliadin being extreme examples. Casein attained maximum ammonification in 6 days, and probably before that time, as has been shown by Kelley,[4] while gliadin had not reached the maximum point after 30 days.

It is noted that a greater percentage of all the different forms of nitrogen is contained in the soluble portions, although before bacterial action very little of either casein or gliadin is soluble. The changes in the different forms of nitrogen, soluble and insoluble, are evident from the tables.

The half of the soluble portion that was not hydrolyzed was used to make determinations of the soluble amino nitrogen. This was accomplished by taking an aliquot part and analyzing by the Van Slyke method, using the small Van Slyke apparatus. Similar determinations were made upon acid-hydrolyzed samples and water solutions of the untreated protein. The water solution was prepared by taking 1 gm. of the protein substance, adding 20 cc. of distilled water, and allowing it to stand 5 hours, shaking at frequent intervals. After allowing to settle for a few minutes, the supernatant liquid was decanted through filter paper.

Table VI shows a comparison of the amount of free amino-acid nitrogen contained in gliadin and casein before and after hydrolysis and after ammonification by the organism *Bacillus subtilis*.

TABLE VI.

	Before hydrolysis.	After hydrolysis.	After ammonification.
	mg.	*mg.*	*mg.*
Gliadin	0.57	42.56	17.03
Casein	0.68	99.31	46.00

We note from these results that there was a very small amount of free amino nitrogen in the water solution of the untreated protein, while in the acid-hydrolyzed and ammonified sample a large amount was obtained. This indicates a similarity of chemical change between acid hydrolysis and bacterial action, and we would therefore infer that in ammonification the protein is hydrolyzed into amino-acids by the action of bacteria.

[4] Kelley, W. P., *Hawaii Agric. Exp. Station Bull. 37*, 1915.

The results obtained thus far do not lead to any definite conclusions relative to the cause of incomplete ammonification of protein substances. All the various forms of nitrogen are present after bacterial action, although necessarily in different proportions. The question naturally arises: Can it be the presence of a toxic substance resulting from bacterial decomposition that tends to inhibit ammonification beyond a certain point? For the purpose of getting light upon this question, sand cultures of casein were prepared as in the previous experiment, sterilized, inoculated with *Bacillus subtilis*, and allowed to incubate until no further ammonification was noticed. The unammonified protein substance was then separated from the sand and a further separation of the insoluble and soluble material, as described above, was effected. These separate parts were then dried on the steam bath, ground to a fine powder in a mortar, introduced into individual flasks containing fresh sand, sterilized, and again inoculated with a fresh culture of *Bacillus subtilis*. Ammonification was allowed to continue first for 2 weeks, after which time a very small amount of ammonia was formed. The flasks were again placed in the incubator and allowed to ammonify for 3 weeks longer when analysis showed that the ammonia continued to be formed only in negligible amounts. During this time, the organism, *Bacillus subtilis*, was the only organism present; and in order to ascertain whether or not other bacteria, as are present in an ordinary soil infusion, would show a greater decomposition of the remaining protein substance, with the ultimate formation of ammonia, the flasks were now inoculated with a soil infusion and allowed to ammonify for 2 weeks. The results indicated a slight ammonification, yielding 2.4 mg. of ammonia for the flask containing the solid material and 2.1 mg. for the other containing the solution. The amount of ammonia formed in 2 weeks is very small, and after 5 weeks' further incubation it increased slightly.

The results indicate that the degree of ammonification is not influenced by the presence or absence of any particular form of nitrogen contained in the protein molecule, nor by any decomposition product which could be destroyed by sterilization, nor by the presence of a toxic substance. If these inferences are true, it is probable that ammonification can proceed only to a point where there occurs a residual portion of the original molecule

which is sufficiently stable to withstand the action of bacteria. Any action that would be sufficiently strong to tear down this residue would in all probability permit further ammonification. Whether this further cleavage of the protein molecule occurs under soil conditions is a matter which can only be determined by further experiment.

SUMMARY.

A limited study has been made on the chemical changes that occur when a protein substance is acted upon by certain organisms.

The results indicate that:

1. All the nitrogen forms are changed more or less by the action of the bacteria, and the end-product ammonia is formed. In no case was one form of nitrogen completely destroyed.

2. The rapidity of action varies greatly with different protein substances, casein showing no further change after a few days, while gliadin continued to ammonify after 30 days.

3. One organism does not act upon different proteins alike. The relative proteolytic activities of the organisms used depend on the proteins acted upon.

4. The monoamino-acid nitrogen and diamino-acid nitrogen of the protein are the chief sources of the ammonia formed by bacterial action.

5. The similarity of chemical change between the action of acid hydrolysis and the action of bacteria indicates that the latter is largely hydrolysis to the point of formation of various amino-acids.

6. There is no toxic substance formed that would inhibit complete ammonification of a protein.

THE EFFECT OF THE INGESTION OF DESICCATED PLACENTA ON THE VARIATIONS IN THE COMPOSITION OF HUMAN MILK DURING THE FIRST ELEVEN DAYS AFTER PARTURITION.

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INTRODUCTION.

In order to arrive at any conception of the factors underlying mammary secretion it is necessary to differentiate between the period of development of the mammæ, the period of their hypertrophy during pregnancy, and the period of secretory activity after parturition. From the standpoint of biochemistry the third period is one of special importance.

The influence of the placenta on the secretion of milk has been the object of attention of several investigators. Fellner (1) and Biedl and Königstein (2) seem to think that repeated injections of placental extract tend to cause hypertrophy of the gland, yet neither Kreidl and Mandl (3) nor Frank and Unger (4) could observe this effect. From the researches of O'Donoghue (5, 6, 7) and of Ancel and Bouin (8) it is evident that the internal secretion of the ovaries is the most important factor in the preparation of the mammary gland for the assumption of its function.

Niklas (9) gives a comprehensive bibliography of the observations on the effect of placenta on milk secretion from the quantitative standpoint. Basch (10), Lederer and Pribram (11), Aschner and Grigoriu (12), and Niklas (9) claim that an increased secretion of milk usually occurred as the result of the injections of placental extract. Fieux (13), Lane-Claypon and Starling (14), Biedl and Königstein (2), Mackenzie (15), and Gaines (16) fail to find such an increase. In fact both Mackenzie and Gaines report an inhibition. Feeding experiments have given similar inconstant results. On women the increase was usually of but slight degree only.

Letulle and Larrier (17) consider that the active substance came from the syncytium, while Ercolani (18), Creighton (19), and Pinoy (20) claim that it is the result of the degenerative changes in the placenta.

As the natural result of these observations there are two sets of ideas regarding the part played by the placenta in milk secretion. The one initiated by Hildebrandt (21) and supported by Halban (22) rests upon the negative results, and considers that during pregnancy the placenta gives off a substance inhibitory to the secretory activity of the mammary gland, but excitatory as regards its hypertrophy, with the complementary idea that the removal of this inhibitory stimulus results in secretory activity. A chalone action is thus attributed to the placental secretion. The other, probably best expressed by Niklas, considers that at delivery there is an overflowing of hormones into the maternal blood, which after a certain period of incubation bring about the initiation of the activity of the milk-producing cells, and which in all probability come from the placenta.

It would appear from the foregoing that a chemical study of the composition of human milk when produced under the influence of placental ingestion would be of some assistance in determining whether or not the activity partakes of the type of a chalone or hormone. It would likewise show in some degree the influence of the placenta upon the maternal metabolic changes preparatory to the taking on of a food-producing function.

In a previous paper (23) a somewhat detailed report was given of the variations in the chemical composition of human milk during the first 11 days after parturition. The present paper is a report of the changes induced in these variations by the oral administration of desiccated placenta.

The subjects of this experiment were healthy patients in the Los Angeles County Hospital. Throughout the period of study the diet was the same for all, and was the same as for those previously reported, with the exception that 0.6 gm. of desiccated placenta was ingested three times a day, commencing with the day of delivery. The milk production of eight women was studied.

Case.	Date.	Case.	Date.
	1916		*1917*
1	Aug. 28–Sept. 5	5	Feb. 10–18
2	" 29– " 6	6	" 13–21
3	" 29– " 6	7	" 13–21
4	" 30– " 7	8	" 25–Mar. 5

The milk was collected by means of the breast pump at the same hour on the 3rd, 5th, 7th, 9th, and 11th, days after parturition.

Methods.

Preparation of the Placenta.—The fresh placenta was ground to a pulp in a meat grinder, mixed with 5 to 10 cc. of toluene, and spread in a thin coat over a large glass plate. It was then dried, without decomposition, in about 48 hours, to a leathery, semi-brittle mass, using the air current from an electric fan. This material was then ground as fine as possible and dried for 24 hours over sulfuric acid in a partial vacuum at room temperature. Regrinding resulted in a fine dry powder of uniform consistency and a not unpleasant odor. This powder was administered in capsules.

Analyses.—The analytical processes used were the same as those given in the report on normal milk secretion (23).

Total Nitrogen and Protein.

Total nitrogen only was determined and the protein calculated from these figures by multiplying by the factor 6.37. With the exception of the 3rd day the ingestion of placenta has the effect of increasing the per cent of protein in the milk about 12 per cent. It apparently causes a lowering of the initial protein per cent in colostrum. The results are recorded in Table I.

TABLE I.

The Percentage of Protein in Human Milk on the 3rd, 5th, 7th, 9th, and 11th Days after Parturition, when Desiccated Placenta Is Fed.

Subject.....	1.	2.	3.	4	5.	6.	7.	8.	Average.	High.	Low.
Day.	Protein.										
3rd	2.11	6.08	3.44	1.90	2.22	2.24	2.32	2.68	2.87	6.08	1.90
5th	1.64	1.85	2.52	1.56	1.82	1.79	2.22	2.09	1.94	2.52	1.56
7th	1.55	1.83	2.87	1.86	1.72	1.60	2.06	1.77	1.91	2.87	1.55
9th	1.41	1.86	2.95	1.90	1.52	1.93	2.12	1.70	1.92	2.95	1.41
11th	1.20	1.82		1.42	1.61	1.38	2.02	1.33	1.54	2.02	1.20
Average.	1.58	2.69	2.95	1.73	1.78	1.79	2.15	1.91	2.04	3.29	1.52

Group Variations for Each Day.—In 88 per cent of the cases on the 3rd day the extreme limit of variation from the mean is ± 29 per cent, which is 40 per cent less variation than that found when no placenta is fed. This limit undergoes a slight narrowing on the 5th day, but from then on expands, so that by the 11th day of lactation the variation from the mean is ±25 per cent, almost equal to the initial limit and nearly 40 per cent greater than normal.

Group Variations from Day to Day.—From the 3rd to the 11th day of lactation there is a general fall in protein production, the most marked decline taking place between the 3rd and 5th days. It should be noted that, as with normal protein production, there is also here an evident tendency towards an increase in protein per cent between the 7th and 9th days. This increase is of sufficient frequency to denote the presence of some important change taking place in the protein production mechanism at this time. Qualitatively, therefore, the ingestion of placenta fails to alter the usual course of production direction. Quantitatively the decrease in production is of a lesser degree than normal, resulting in a uniformly higher protein per cent in the milk of women ingesting the desiccated placenta.

The Effects of the Ingestion of Desiccated Placenta on the Variations in the Protein Percentage of Human Milk.—The feeding of desiccated placenta to women during the first 11 days of lactation decreases the per cent of protein in colostrum; it increases the production from then on about 12 per cent above the normal; it causes a narrowing of the limits within which this production may be supposed to occur in colostrum, with an evident later expansion of these limits above those normally found; it does not alter the normal changes in production direction at any time; it has practically no effect on the individual variability from day to day; and the plane of protein production remains nearly as uniformly above or below the group average for the single days as when no placenta is ingested.

Fat.

In Table II will be found the per cent of fat in the milk of eight women on the 3rd, 5th, 7th, 9th, and 11th days after parturition, when desiccated placenta is fed.

As a whole, the effect of the ingestion of desiccated placenta on the fat per cent in human milk is the opposite of that produced on the protein. It causes a tendency towards an increase in the per cent of fat in colostrum, with a decrease in the succeeding days, when compared with the milk produced by individuals not subjected to its influence.

The Effects of the Ingestion of Desiccated Placenta on the Variations in the Fat Percentage of Human Milk.—The feeding of desiccated placenta to women during the first 11 days of lactation tends to decrease the per cent of fat in milk; it causes an expansion of

TABLE II.

The Percentage of Fat in Human Milk on the 3rd, 5th, 7th, 9th, and 11th Days after Parturition, when Desiccated Placenta Is Fed.

Subject.....	1.	2.	3.	4.	5.	6.	7.	8.	Average.	High.	Low.
Day.	Fat.										
3rd	2.26	7.06	3.32	4.14	3.72	2.88	4.88	6.53	4.35	7.06	2.26
5th	1.44	2.94	1.89	2.57	3.89	3.08	4.80	2.30	2.58	4.80	1.44
7th	2.10	3.30	4.41	2.59	2.14	3.69	8.88	2.67	3.72	8.88	2.10
9th	4.09	2.36	3.10	1.74	1.93	2.46	4.84	3.52	3.00	4.84	1.74
11th	2.72	3.98		2.71	2.63	3.62	6.30	4.17	3.73	6.30	2.63
Average.	2.52	3.93	3.18	2.75	2.86	3.15	5.94	3.84	3.48	6.38	2.03

the limits within which the production may be supposed to occur in colostrum; it causes a later narrowing of these limits below those normally found; it tends to regulate the variation in production direction; it increases the individual variability to a marked extent; and it causes the plane of fat production to tend towards uniformity to a greater degree than is found in the milk of individuals not subjected to its influence.

Lactose.

In Table III will be found the per cent of lactose in the milk of eight women on the 3rd, 5th, 7th, 9th, and 11th days after parturition, when desiccated placenta is fed.

TABLE III.

The Percentage of Lactose in Human Milk on the 3rd, 5th, 7th, 9th, and 11th Days after Parturition, when Desiccated Placenta Is Fed.

Subject.....	1.	2.	3.	4.	5.	6.	7.	8.	Average.	High.	Low.
Day.	Lactose.										
3rd	6.49	6.37	5.74	5.16	4.47	5.73	5.88	5.83	5.71	6.49	4.47
5th	7.00	6.20	6.49	6.81	5.47	6.12	6.26	6.63	6.37	7.00	5.47
7th	7.19	7.00	4.05	7.03	6.56	6.44	5.98	6.56	6.35	7.19	4.05
9th	7.53	7.14	5.58	7.20	6.66	6.69	5.75	5.98	6.57	7.53	5.58
11th	7.37	7.03		7.24	6.86	6.86	6.01	5.85	6.75	7.37	5.85
Average.	7.12	6.75	5.47	6.69	6.00	6.37	5.98	6.17	6.35	7.12	5.08

A comparison of the per cent of lactose found in the milk of women during the first 11 days of lactation, with and without the ingestion of placenta, will demonstrate that in the former there has been a consistent increase in sugar production.

The Effects of the Ingestion of Desiccated Placenta on the Variations in the Lactose Percentage of Human Milk.—The feeding of desiccated placenta to women during the first 11 days of lactation causes an increased activity of the lactose-producing mechanism with the consequent generally higher sugar per cent in the milk; it does not alter to an appreciable extent the variability of the limits within which this production may be supposed to occur; although decreasing the individual variability, it seems to cause a slight instability of the usual normal uniformity of change in production direction, an effect opposite to that observed on fat; it causes a diminution of the normal tendency towards a uniformity of plane of production, again different from what occurs with fat.

Variations in the Constituents.

With the decrease in the plane of production for fat there is an increase in the plane of production for both protein and lactose when these results are compared with those obtained during normal milk secretion. The reciprocal relationship existing between fat and lactose production in normal milk when calculated from the individual per cent variation from day to day and the per cent change in production direction is not as well marked under the influence of ingested desiccated placenta. This is due in part to the decrease of the uniformity of change in production direction, and in part to the decrease in per cent variability from day to day, of the lactose production. The alteration in the plane of production of these two constituents seems to have a tendency towards compensating their individual reciprocal variability, which, however, does not in the least signify that they are not still mutually interdependent.

SUMMARY.

In comparing the variations in the fat, protein, and lactose per cent of milk produced during the first 11 days after parturition, when desiccated placenta is fed, with the variations observed

in these constituents for a similar period in normal milk, it is seen that there are some significant differences. Quantitatively the plane of production for lactose and protein has been raised, while that for fat has been slightly decreased. The general change in production direction remains the same.

When we consider as an index of variability the individual variation per cent from the group average for the single days and the individual variation per cent from day to day, the constituents fall into the same order of decreasing variability as in normal milk production; *fat, protein, lactose.* But when the group changes in variation limits and the group uniformity of change in production direction are considered, the order changes from *fat, lactose, protein,* as found in normal milk, to *lactose, fat, protein,* with but slight differences between them.

The tendency for the production plane to be fixed for the individual and independent of the plane of nutrition is here maintained, though to a slightly less extent.

CONCLUSIONS.

The results recorded in this paper demonstrate that the ingestion of desiccated placenta has an effect upon the factors concerned in the regulation of the chemical composition of milk. There is a stimulation of the sugar- and protein-producing mechanism with an apparent depression of the function of the fat-secreting apparatus.

From the peculiar characteristics of milk protein and carbohydrate it is presumable that these constituents are largely elaborated by the gland itself.

There is certain evidence pointing to the idea that the milk fat is not entirely elaborated in the mammary gland. In working with goats, Winternitz (24, 25) and Bowes (26) have shown that probably a part of the ingested fat is carried as such to the mammary gland and then by excretion becomes a milk constituent. Eckles and Palmer (27, 28) clearly show that for the cow there is a variation in the nature and composition of milk fat dependent in some degree upon the diet. The probability of there being present in the gland a specific fat production mechanism for the elaboration of the fats peculiar to milk cannot be denied even though Bradley (29) failed to detect the presence of a lipase in

this tissue. It would therefore appear that milk fat is the sum total of the secretory and excretory activities of the mammary gland, the former being concerned with the elaboration of the fat peculiar to milk, the latter concerned in the inclusion in the milk of a part of the ingested fat as such. The evidence for this is admittedly incomplete.

From the fact that the ingestion of desiccated placenta tends to produce a milk of greater uniformity in change of production direction of fat, it does not seem improbable that its action may well be stimulative to the secretory activity of the gland in this respect also. The cause of the lower per cent of fat in the milk may indeed be founded either in a decreased absorption of ingested fat or in a decreased fat excretory activity of the gland.

These findings seem to lend support to the hypothesis of Niklas (9).

No chalone activity is evident.

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STUDIES ON THE THEORY OF DIABETES.

VIII. TIMED INTRAVENOUS INJECTIONS OF GLUCOSE AT LOWER RATES.*

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INTRODUCTION.

Principles underlying the method of timed intravenous injections at known rates, and an improved pump designed for the purpose were recently described.[1] Their application in a clinical study of glucose tolerance has been reported elsewhere by Wilder and Sansum.[2] This paper deals generally with the technique of the experiments carried out on rabbits and dogs, and, in particular, with observations concerning the effects of sustained intravenous injections of glucose at rates up to 2 gm. per kg. per hour. The experiments with higher rates of injection will be made the subject of another paper. Apart from recording certain data concerning the behavior of glucose and water, the purpose of this paper is further to discuss the relative importance of rate and concentration measurements in biological work generally.

Methods.

Material.—The pure glucose used in the majority of experiments was prepared in the laboratory as described below from a completely hydrolyzed granular commercial glucose from cornstarch.[3] A Kahlbaum c. p. glucose preparation was used at in-

* A third detailed report on phases of the work outlined by Woodyatt, R. T., Sansum, W. D., and Wilder, R. M., *J. Am. Med. Assn.*, 1915, lxv, 2067.

[1] Woodyatt, *J. Biol. Chem.*, 1917, xxix, 355.

[2] Wilder and Sansum, *Arch. Int. Med.*, 1917, xix, 311.

[3] Made at the Argo plant of The Corn Products Refining Company.

tervals for control purposes. Some lots of the product made in the laboratory caused melituria when injected at rates below that found necessary with the Kahlbaum preparation.

1,000 gm. of the crude glucose are dissolved in 400 cc. of boiling water digested 1 to 2 hours (not longer) with animal charcoal and the solution filtered hot by suction. Add 800 cc. of 95 per cent alcohol, cool, and inoculate with glucose crystals. The first crystals (5 to 10 gm.) carry down suspended carbon and are filtered off. As further crystallization proceeds, filter off successive fractions to avoid caking. Wash the crystals with cold alcohol on a suction funnel. (Save the alcoholic washings to dilute the next concentrated crude glucose filtrate.) Yield = 50 to 60 per cent. Repeat the process if necessary. If the crude glucose employed contains much tarry matter, it may be necessary to use more than 400 cc. of water per 1,000 gm. at the outset to obtain a filterable solution. The final pure white product was made up into 1, 2, 3, and 4 molecular (18, 36, 54, or 72 per cent) solutions with water freshly distilled as for a salvarsan injection and placed in 2 to 3 liter flasks in which they showed only a straw or light yellow color. This color may be wholly removed by a final clearing with a little bone-black. After sterilization and before use, the exact strength of each solution was determined by polariscope checked at times by a reduction method. Solutions of 1 and 2 molecular strength proved most generally useful.

It may be noted that a 1 gm. molecular or 18 per cent aqueous glucose solution contains, at 17.5°C. per 100 cc. volume, 89.44 gm. of water (Baling) and 18 gm. of glucose, or 1 molecule of glucose to 50 molecules of water, whereas a 2 gm. molecular (36 per cent) aqueous glucose solution contains, per 100 cc., 79.90 gm. of water and 36 gm. of glucose, or 1 molecule of glucose to about 22 molecules of water.

The subjects were dogs, rabbits, and human beings. The bulk of the work was with dogs weighing from 9 to 19 kg. Care was taken to select vigorous animals of gentle and phlegmatic dispositions received from active outdoor life. Bull terriers or mongrels with a strong admixture of bulldog blood were used in most of the satisfactory experiments, whereas most of the failures occurred with unhealthy, housed, ill fed, or nervous animals.

Technique.—The technique of the experiments as applied to human subjects was the same as that described and illustrated by Wilder and Sansum[2] in their report on clinical glucose tolerance determinations by the intravenous method. The routine of the dog experiments requires further description.

The solution to be injected is poured into a previously sterilized burette of about 500 cc. capacity. The burette has a glass cock and the tip connects by rubber tubing with one arm of a glass Y-tube from the stem of

which rubber tubing leads to the suction nipple of a volumetric pump. A second slender burette beside the first connects with the other arm of the Y-tube and serves for short interval readings. Half hourly or hourly rates are checked on the reservoir burette. One or two coils of the rubber tubing which carries the solution from the burettes to the pump are allowed to rest in a water bath to warm the sugar solution. The delivery tube from the pump terminates in a small cannula inserted into a vein, usually at the ankle in the case of dogs. With rabbits the injections have been through a small needle inserted into the ear vein and held by a small clamp. In many of the experiments two pumps have been employed, the second or auxiliary taking distilled water from a second pair of burettes and discharging it through a delivery tube and a needle which is thrust obliquely through the wall of the rubber delivery tube of the main pump; or, the two delivery tubes may be permanently anastomosed. Thus it is made possible *to control the concentrations of water and glucose as well as the absolute quantities in which they are injected.*

The dogs lie on their backs or partly on the side in an ordinary animal trough cushioned with blankets. There is a leather collar about the neck and a not too snug jacket or flannel bandage on the chest. Collar and jacket are secured below to the animal board allowing limited freedom of movement, care being taken not to impede respiration. The fore legs are free. The hind legs are separated to the opposite lower borders of the board and held firmly by slings but not in such a way as to cause pain or stop circulation.

A catheter is placed in the bladder and secured by a strip of adhesive tape fixed to the catheter and carried upward on to the abdomen. It was found desirable to accustom the dogs to these procedures in sham experiments on previous days before attempting long injections. Preferably, exposure of the vein has been carried out under novocaine on the day before the experiment, in which case ligatures have been laid, the wound smeared with vaseline, and the whole enclosed with a band of adhesive tape. In the experiment the cannula may be inserted without causing pain. These precautions were taken to avoid non-physiological factors and because experience proved that restlessness, discomfort, pain, or emotion could affect the results, especially the rate of water secretion. In many experiments the laden animal board has been made to rest on the platform of a scales and the scale beam so balanced that gains or losses of body weight incident to the development of positive or negative water balances could be detected and, if desired, corrected by changing the rate of the water injection by means of the auxiliary pump.

The animal experiments have all been conducted in a separate room from which noise and confusion could be excluded. In satisfactory experiments dogs have lain quietly for many hours at a time, the pumps have required little attention, and the collection of urine has proceeded automatically except for required manipulation at the end of the hourly periods.

EXPERIMENTAL.

As an aqueous solution of glucose may be regarded as representing merely so many molecules of glucose, so many of water, or as glucose and water together, each affecting and affected by the other in a way not fully understood, to form among other things a series of hydrates and their dissociation fragments in accordance with the solvate theory,[4] so intravenous injections of aqueous glucose solutions and their physiological effects have to be considered from at least these viewpoints. Although in this report attention is centered on the glucose this necessarily involves some consideration of the corresponding movements of water into and through the body.

Continued Injections of Glucose into the Systemic Blood at Rates Not Greater Than 1.0 Gm. per Kilo of Body Weight per Hour.

In all, upwards of fifty experiments have been conducted in man, dogs, and rabbits, receiving glucose by vein at the lower rates. Conditions were considered standard when the subjects were healthy, well nourished, mature individuals in a state of complete bodily rest and free of pain, discomfort, emotion, or other tangible nerve-disturbing influence, when the glucose employed reacted chemically and physiologically as pure *d*-glucose and when the solutions employed varied in strength between 16 and 40 per cent. The gm. molecular (18 per cent) solution was most commonly used. The duration of the experiments varied from ½ hour to 7 hours.

In man, injections at the rate of 0.8 gm. per kg. per hour were not observed to produce glycosuria detectable by the ordinary test solutions employed (Fehling's, Haines'), after 30 minutes to 3 hours of injection. *In dogs and rabbits* the same results were obtained. One dog received glucose at this rate for 7 hours with the same result. Injection at the rate of 0.9 gm. per kg. per hour *in man* uniformly caused glycosuria, also *in rabbits and dogs* in the great majority of cases but not in all. In four dogs it required 1.0 gm. of glucose per kg. per hour to cause glycosuria. It

[4] Cf. Jones, H. C., Absorption spectra of solutions as studied by means of the radio-micr meter, *Carnegie Institution of Washington, Publication No. 210*, 1915. A summary of earlier literatures is given at the end.

has been observed that glycosuria has failed to develop when the injection rate has been between 0.8 and 0.85 gm., and appeared when the rate has been advanced to between 0.85 and 0.90 gm. per kg. per hour.

The time elapsing between the beginning of an injection at the 0.9 gm. rate and the detection of glycosuria was always short when this rate was instituted directly after ½ hour or more of injection at the rate of 0.6 to 0.8 gm. per kg. per hour, and longer when the 0.9 gm. rate was instituted with no prior glucose administration. Owing to the time necessary for enough urine to collect in the bladder, to remove and test satisfactorily, the exact interval elapsing between the beginning of the 0.9 gm. rate and the actual secretion of saccharine urine by the kidneys cannot be stated, but it was found to be as long as 40 minutes with one dog which had received no prior administration, and as short as 6 minutes in a dog which had received glucose for ½ hour at the 0.8 gm. rate before the rate was advanced. Following preliminary injections at a subtolerant rate for ½ hour or more, an advance to the 0.9 rate has regularly caused sugar to appear in the urine within the first half hour.

Conclusions.—On the basis of all the experiments taken collectively it is reckoned that the maximum rate at which glucose can be administered into the systemic blood and still fail to produce glycosuria in normal resting human individuals is close to 0.85 gm. per kg. per hour. Perhaps in dogs and rabbits it averages a little higher than in man. (These statements agree in the main with those made in our preliminary report on the basis of experiments then completed. They take into account the later series of observations on human subjects described by Wilder and Sansum, and further dog and rabbit observations which permit the correction of slight inaccuracies in the earlier report.)

The Behavior of Water in Conjunction with Sustained Intrasystemic Glucose Injections at Subtolerant Rates.

Two things may be noted here: (1) The same results as described above have been obtained with glucose solutions varying in concentration between 16 and 40 per cent. The method em-

ployed did not permit the detection of any difference of the intravenous glucose tolerance limit as a result of variations between rather wide limits of the quantity of water which accompanied the glucose into the vein. Thus in dogs weighing 10 kg., the limit was found at 0.85 gm. per kg. per hour both with 47 cc. of 1 M (18 per cent) and with 23.5 cc. of 2 M (36 per cent) glucose solutions. Even when solutions containing 72 to 100 gm. of glucose per 100 cc. have been used, the tolerance limit has not varied by degrees which could be detected by the methods employed. The same has held for solutions as weak as 9 per cent. However, the more concentrated solutions increase the difficulties of maintaining exact injection rates when dealing with dogs and rabbits, and following an injection of 72 per cent solution in one human case, a thrombus developed in the vein so that no further use was made of such strong solutions in the clinic. Too dilute solutions, on the other hand, may introduce such factors as hemolysis and significant changes of weight. (2) During intravenous glucose injections at subtolerant rates there has been observed a strong tendency toward retention of water in the organism, which under appropriate experimental conditions may be manifested by an absolute decrease in the rate of diuresis that appears after the injection has been continued for a sufficient length of time, lasts during the remainder of the injection, and promptly gives way to a sudden increase of diuresis when the injection is stopped. The following experiment is typical.

EXPERIMENT I.

The Retardation of Diuresis during Intravenous Injection of Glucose at a Subtolerant Rate with High Diuresis after Injection.

A dog weighing 12.7 kg. received 40 cc. of water *per os* at 8 and again at 9 a.m. when it was placed on the board, catheterized, and prepared for intravenous injection. Thereafter the bladder was emptied by catheter every hour. The injection consisted of 51.5 cc. of 19.7 per cent glucose solution (containing about 45 cc. of water) per hour. Rate, 0.8 gm. glucose per kg. per hour. The table shows the amounts of urine obtained at the end of each succeeding hour, the bold-faced figures indicating hours of injection.

Hour.	Urine.	Remarks.
	cc.	
10 a.m.	40	
11 "	30	
12 "	**13**	Injection 0.8 gm. per kg.
1 p.m.	**18**	" 0.8 " " "
2 "	**7**	" 0.8 " " "
3 "	**8**	" 0.8 " " "
4 "	**9**	" 0.8 " " "
5 "	**10**	" 0.8 " " "
6 "	**12**	" 0.8 " " "
7 "	80	

Of the 80 cc. of urine passed between 6 and 7 o'clock, 58 cc. appeared in the first 25 minutes. Thereafter for 12 hours the average urinary volume was 12 cc. per hour. It may be noted that the intake of water per hour during injection was about that given in the fore-periods. There was no glycosuria.

We have failed to observe such absolute retardations of diuresis in experiments in which the quantity of water given during the preparatory periods or during the glucose injection periods has been too great. In such cases, however, there has never been noted any increase of diuresis attributable to the glucose itself; *i.e.*, the injection of glucose solutions at subtolerant rates has not been observed to elevate the diuresis more than the injection of equivalent volumes of water in the form of 0.6 to 0.8 per cent sodium chloride solutions. It would appear that in the presence of an excess of water in the organism, the binding of some by the glucose may fail to lessen the absolute rate of diuresis. Such a negative effect is illustrated by the following experiment.

EXPERIMENT II.

The Absence of Retardation of Diuresis during Intravenous Injection of Glucose at Low Rates.

The weight of the dog was 14.3 kg. At 8, 9, 10, and 11 a.m. it received by stomach tube 70 cc. of water (total 280 cc.). At 1 p.m. the bladder was emptied by catheter and thereafter every hour. Injection was begun at 2 p.m. In the 1st hour the rate was 0.8, in the second 0.9, in the third 1.0 gm. per kg., glycosuria appearing 10 minutes after beginning the latter rate. The bold-faced figures show hours of injection. The rate of water supply was the same during the preparatory and the injection hours.

Hour.	Urine.	Remarks.
	cc.	
2 p.m.	44	
3 "	39	Injection 0.8 gm. glucose per kg.
4 "	48	" 0.9 " " " "
5 "	52*	" 1.09 " " " "
6 "	48	

* Glycosuria in the 3rd hour.

Both types of result have been observed during intravenous glucose injections in man, but owing to the undesirability of catheterizing normal individuals for a sharp separation of the urine at short intervals, the greater frequency of psychic and nervous influences, and other factors which make it difficult to secure uniform rates of diuresis during the control periods, observations of diuresis in man have been unsatisfactory. (Although Pavy[5] had described retardation of diuresis during low rate intravenous injections of glucose, we had not succeeded in confirming his observation at the time of our preliminary report.)

Injections at Rates between 1 and 2 Gm. of Glucose per Kg. per Hour.

With injections of 1.8 to 2.0 gm. per kg. per hour glycosuria begins promptly and quickly attains a maximum, after which it proceeds at a uniform rate hour after hour for at least 8 hours and probably much longer. Reference to the record of Experiment III, which is typical, will show that the total excretion during the 1st hour may equal that of later hours, indicating the promptness with which the maximum glycosuria may be established with an injection at this low rate.[6]

The absolute rate of the glycosuria may differ in different individuals, and probably in the same individual under different physiological conditions.

In Experiment III the average excretion was 0.42 gm. per kg. per hour, or 2.1 per cent of the intake. Excretions representing

[5] Pavy, F. W., *J. Physiol.*, 1899, xxiv, 479.

[6] With higher rates of injection (2.7 to 7.2 gm. per kg. per hour) the glycosuria gradually accelerates during the first 1 to 5 hours or more before constancy is finally attained, the time required depending largely on the rates of glucose administration and on the water balance.

about 2 per cent of the intake have been seen in other experiments, but one dog receiving 1.8 gm. of glucose per kg. per hour excreted 1.90, 1.75, 2.04, 1.62, 1.61, and 1.97 gm. of glucose per 10 kg. in successive hours, an average of 1.8 gm. per hour representing 10 per cent of the injection.

The glycosuria has been observed to cease within 6 minutes after stopping the injection; *i.e.*, if the bladder is washed out immediately after ending the injection, the next urine collected has been found free of sugar.

The Behavior of Water during Injections at Rates of 1.8 to 2.0 Gm. per Kg. per Hour.

1. Within rather wide limits the quantity of water given before an experiment or during the glucose injection may be varied without influencing the rate of glucose excretion. This is typically illustrated in the following experiment.

EXPERIMENT III.

The Constant Supply and Constant Excretion of Glucose While the Supply and Excretion of Water Varies.

The subject was a bull terrier weighing 16.7 kg., which had served in four previous experiments of similar character. Two pumps, with the delivery tubes anastomosed, discharged water and 37.01 per cent glucose solution respectively through a single cannula. The pump discharging glucose solution ran at a constant rate to deliver 53.9 cc. per 10 kg. of body weight per hour (containing 20 gm. of glucose and 42.6 cc. of water). The second pump delivered water at rates which were varied between 53.9 and 107.8 cc. per kg. per hour. The rates of injection and elimination of glucose and water per 10 kg. of body weight are tabulated by hours from the beginning of the injection.

Injected.			Excreted.		
Hour.	Glucose.	Water.	Glucose.	Urine.	Urinary glucose concentration.
	gm.	*cc.*	*gm.*	*cc.*	*per cent*
1	20	96.5	0.43	16.8	2.57
2	20	96.5	0.31*	6.2	4.90
3	20	151.5	0.52*	59.8	0.87
4	20	123.5	0.44	118.3	0.37
5	20	96.5	0.42	128.0	0.35
6	20	96.5	0.44	82.5	0.59
7	20	96.5	0.41	58.5	0.62
8	20	96.5	0.42	67.0	0.58

* Average 0.42 gm.

In this experiment it will be noted that in the 2nd hour when the urine was only 6.2 cc. and contained nearly 5 per cent of glucose, the excretion was 0.1 gm. below the average of the 1st hour and the last 5 hours, whereas in the 3rd hour when the urine rose to 58.9 cc. it was a trifle above. But the average of the 2nd and 3rd hours was 0.42 gm., the same as for the others, and this fluctuation would be accounted for if only a 2 cc. residuum of the concentrated urine had remained in the bladder, urinary passages, and kidneys to be flushed out during the diuresis of the following hour. There might of course be some retention in the tissues also if the supply of water was too small. In the 7th hour, with the same urinary volume as in the 3rd, the excretion of glucose was 0.41 gm. These observations parallel those made with injections at the 0.8 to 0.9 gm. rates and imply that the glucose utilization rate, as well as the excretion rate, may remain the same during marked fluctuations of the water balance.[7]

2. Unlike injections at subtolerant rates, those productive of glycosuria, may, as is well known, cause acceleration of diuresis. But as in the case of the former, the more rapid injections may be attended by no absolute change of the rate of diuresis, or they may lead to absolute retardations. Which effect will occur appears to depend wholly on the supply of available water in the organism and on the rate of the glycosuria, other factors remaining constant. In experiments with injections at these rates it is possible, under suitable conditions, to demonstrate diminished diuresis during the injection of 1.8 to 2.0 gm. of glucose per kg., followed by sudden liberation of water into the urine when the injection is stopped, exactly as in the case of subtolerant injections; only to do so the water balance must be kept at a relatively lower level.

Frequently in the course of clinical work with 18 per cent glu-

[7] With higher rates of glucose injection, and with wider variation of the water balance at the present rates, the excretion may be affected. However, these observations would not be in harmony with A. A. Epstein's statement (Studies on Hyperglycemia in Relation to Glycosuria, New York, 1916, 69) that "diuresis in diabetes plays an important rôle in determining the total amount of sugar eliminated in the urine, but has no influence on its concentration." They agree, on the other hand, with the finding of T. Addis and C. K. Watanabe (*J. Biol. Chem.*, 1917, xxix, 404) that "changes in the volume of the urine or in the urea concentration of the urine have no appreciable effect on the rate of urea excretion."

cose solutions, an overstepping of the tolerance has been attended by increased diuresis. With the same solution in dogs, decreased diuresis has at times been observed even when a considerable glycosuria has been in progress, as illustrated in the 2nd hour of Experiment III, but the effect is better shown by experiments in which solutions of 50 per cent concentration or above have been used, as in the following.

EXPERIMENT IV.

Retardation of Diuresis during Intravenous Injection at a Rate Productive of Glycosuria, with Acceleration of Diuresis after Injection.

The dog weighed 17 kg. During the 12 hours preceding the experiment it received no food, but water was provided in the cage. At 8.30 a.m. 55 cc. of water were given by stomach tube and the dog was placed on the animal board and prepared for intravenous injection. At 9.30 it was catheterized and thereafter the urine was drawn by catheter every hour. Intravenous injection was begun with 53 per cent solution at the rate of 1.7 gm. of glucose per kg. per hour; *i.e.*, 55.1 cc. of the solution per hour. The injection was continued for the next 4 hours, ending at 4.30 p.m. At 1.30 the urine contained sugar. At 4.30 it contained 4.4 per cent (total 0.35 gm., representing 2 per cent of the intake).

Hour.	Urine volume.	Remarks.
	cc.	
10.30	32	
11.30	15	
12.30	**15**	Injection started.
1.30	**10**	
2.30	**8**	
3.30	**13**	
4.30	**8**	Injection ended.
5.30	65	
6.30	98	
7.30	20	

During the following 12 hours the average urination was 18 cc. per hour.

In this experiment the retardation of diuresis during the injection is comparable to that seen with injections at subtolerant rates. The high diuresis following the discontinuance of injection is even more pronounced and of longer duration.

The highest concentration of glucose which has been observed in the urine during the present experiments with injections at rates below 2 gm. per kg. per hour has been 8.5 per cent.

It may be stated in anticipation of later papers that the urinary glucose concentration, even with injections at rates as high as 7.2 gm. per kg. per hour, has not been driven higher than 8 to 9 per cent except in the case of a few animals. Since in pancreatic and phlorhizin diabetes concentrations of 12 to 13 per cent are easily produced, the point may have theoretical significance.

DISCUSSION.

The Maximum Rate at Which Glucose Can Be Administered Continuously via the Systemic Blood Stream and Fail to Cause Glycosuria.

The value of 0.85 gm. per kg. per hour agrees only approximately with the findings of Blumenthal[8] and others who have used his method (Comessatti,[9] and Loeb and Stadler[10]). Blumenthal made repeated single injections into rabbits with a hand syringe at 15 minute intervals, each injection consuming 10 to 15 seconds during total periods as long as 3 hours. The tolerance limit in dogs (originally expressed in gm. per kg. per $\frac{1}{4}$ hour, and here recalculated for the hour basis) was found as low as 0.6 and as high as 1.3 gm. per kg. The present experiments lasting as long as 7 hours with consistent results varying with few exceptions only between 0.8 and 0.9 gm. define the normal limit more sharply.

The Significance of the Observation That, within Limits, the Quantity of Water Administered before or during Sustained Glucose Injections at Fixed Rates Need Not Affect the Rate of Glucose Utilization or Elimination.

Whatever variations of the blood and tissue volumes and of the blood and urinary sugar concentrations were involved in the fluctuations of the water balance, they did not noticeably affect

[8] Blumenthal, F., *Beitr. chem. Phys. u. Path.*, 1905, vi, 329.

[9] Comessatti, G., *Beitr. chem. Phys. u. Path.*, 1907, ix, 67.

[10] Loeb, O., and Stadler, H., *Arch. exp. Path. u. Pharm.*, 1914, lxxvii, 326.

the rate of glucose metabolism or excretion as long as the rate of glucose supply was constant. Epstein,[11] working with diabetics, also found that the quantity of glucose excreted might be the same in cases showing different blood sugar concentrations and *vice versa*. Such an effect is strikingly illustrated by an unpublished experiment of our own in which a dog received glucose by vein at the rate of 36 gm., and then 27 gm. per 10 kg. per hour, while the water administration was voluntarily varied with a second pump. In the last hour of the faster glucose injection the excretion was 15.2 gm.; in the 1st hour of the reduced rate of injection, it was 10.23 gm., but the blood sugar concentration in both hours was 0.89 per cent. The occurrence of such phenomena is of course tacitly implied in Ambard's attempts to formulate laws governing them. They are also in keeping with the older observation that the drinking of water and passage of urine need not affect the protein metabolism or the total heat production of the organism, as illustrated specifically by a calorimeter experiment of Lusk's[12] in which an 8.8 kg. dog received 200 cc. of water by stomach with no influence on the metabolism. *But the question arises as to how water may dispose itself in the organism to permit such results and how their recognition bears on current conceptions of the significance of measurements of the concentration of various substances in the blood.*

It is clear that the rate of supply to a given organism at a given time is the chief factor which determines the velocity of chemical change and the rate of elimination, but the mechanism is less obvious. Variations of the total quantity of a glucose solution of fixed concentration on one side of an ordinary dialyzer would not vary the rate at which glucose molecules diffused through it, nor could the concentration be changed *without* affecting the rate of diffusion. But with the kidney membrane and cells in general it is not merely the concentration which determines the rates of excretion and utilization respectively. This would suggest that the surfaces of contact between the plasma and the cells must vary in a definite manner with the volume and glucose concentration of the plasma.

[11] Epstein;[7] see also *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 67, and Epstein, A. A., and Baehr, G., *J. Biol. Chem.*, 1914, xviii, 21.

[12] Lusk, G., *J. Biol. Chem.*, 1912–13, xiii, 41 and 45.

The following, in which cells and plasma represent phases of a system, serves for illustration. If a single spherical cell such as a lymphocyte is suspended in plasma containing glucose, then (when a state of equilibrium has been approached) the velocity at which glucose molecules strike and penetrate its surface, and therefore the velocity at which they react chemically with substances in the cell interior, will, other factors remaining the same, be determined by the concentration of glucose in the plasma and the area of the surface of contact between the cell and the plasma. If the concentration of glucose in the plasma were decreased, by increasing the volume through the addition of water, and *if the cell surface remained unchanged,* this would lessen the frequency with which glucose molecules impinged on the cell, and entail a proportional decrease of the number entering in a unit of time, etc. In like manner, abstraction of water from the plasma with a decrease of its volume and increase of the glucose concentration would increase the rate at which glucose molecules entered the cells and reacted within it. In either of these hypothetical cases the volume change in the plasma would involve changes in the rate of the glucose metabolism of the cell, contrary to the experimental findings under discussion. If, however, as the plasma volume increased or diminished (and the glucose concentration varied correspondingly) the surface of contact between the cell and the plasma increased or diminished in the same proportion, then the total number of glucose molecules impinging on the total cell surface would theoretically remain the same and such an occurrence would explain the experimental findings.

The necessary condition would be fulfilled if while the total content of glucose in the plasma remained fixed and the volume of plasma varied, the ratio of the plasma volume to the surface of contact between the plasma and the cell remained constant; that is, in the body taken as a whole, if *the surface of contact between the plasma and cell phases varied directly as the plasma volume or inversely as the plasma glucose concentration.*

These observations are in harmony with those of Epstein concerning the ratio of the plasma volume to the blood cell volume, the blood sugar concentration, and the rate of glycosuria in diabetics, which led to the conclusion that the total sugar content of the blood fixes the rate of glycosuria. Other things being equal,

it should do so in accordance with the above, *as long as the specific surface of the plasma remains fixed.*

As to how the surface of contact between the plasma and the cells of the body may be regulated to meet changing volumes of blood, several ways are apparent. In the histological sense, the single leukocyte suspended in plasma may increase in volume as water is added to the plasma, or diminish when glucose is added. Dilution of the plasma may separate cells in contact, concentration permit separate cells to cluster together. The wall of the capillary may be more or less distended, the glomerular tuft may inflate and deflate, providing more or less surface in proportion to the blood volume. Or the question may be considered in terms of phases in colloid systems, and the influence of varying contents of sugar and water on their volumes and specific surfaces. As Henderson[13] recalls, Willard Gibbs proved that when equilibrium is established the state of the system is unaffected by the volume of the phases.

Acceleration and Retardation of Diuresis Resulting from Intravenous Injections of Glucose.

The phenomenon of decreased diuresis during injection, with a sudden liberation of water into the urine when the injection is ended, is quite analogous to that observed as a result of alimentary glucose administration by Lusk[12] and Fisher and Wishart.[14] The analogy is made striking by a recalculation of their data, which shows that glucose actually entered the blood by absorption during periods of 2 to 5 hours at mean rates of 1.6 to 1.7 gm. per kg. per hour, as compared with direct injections of 0.8, 1.8, and 2.0 gm. per kg. per hour in the present series.

Fisher and Wishart administered 50 gm. doses of glucose by stomach to dogs weighing 8 to 9 kg., sacrificed them at hour intervals, and found that absorption was completed "during the 4th hour." If the absorption time was 3.5 hours and the average body weight 8.5 kg., this would imply a mean absorption rate of 1.68 gm. per kg. per hour. Lusk found that 50 gm. of glucose might cause a 20 per cent increase in metabolism ending "*in the 4th hour,*" and 75 gm. might also cause a 20 per cent increase

[13] Henderson, L. J., *Proc. Nat. Acad. Sc.*, 1916, ii, 654.

[14] Fisher, G., and Wishart, M. B., *J. Biol. Chem.*, 1912–13, xiii, 49.

which continued "*through the 5th hour.*" As Lusk states, Fisher and Wishart showed "that there is a large retention of water by the organism during the period of high metabolism, which water is suddenly eliminated during the last hour of the high metabolism." In the case of the 50 gm. doses the period of high metabolism ended in the same hour that absorption was completed. From the present experiments it is apparent also that the period of high diuresis comes promptly when the flow of glucose into the blood is ended. Therefore, no doubt, as Lusk assumed, the end of the high metabolism and the onset of high diuresis following the administration of 75 gm. of glucose also marked the end of absorption as directly observed in the case of the smaller doses. Then taking the dog's weight at 7.3 kg.[15] and the time as 5 hours, the rate of absorption would have been 1.6 gm. per kg. per hour as against 1.7 gm. reckoned in the case of the 50 gm. doses. The fact that the metabolism was increased to the same level with the 50 and 75 gm. doses in the same animal confirms the reckoning which shows that the same absorption rate prevailed in the two cases.

Interpretation of the Effects of Glucose on the Water Balance.

The retention of water was attributed by Lusk and by Fisher and Wishart to the increased osmotic power of the organism due to the presence of increased amounts of freely diffusible sugar. Following the alimentary administration of 50 gm. of glucose in 150 cc. of water they found during the 1st hour a rapid absorption of glucose into the blood but no fall of the hemoglobin percentage to indicate hydremia. It was presumed that in this hour the balance of glucose still in the alimentary tract held water back. But by the end of the 2nd hour, two-thirds to three-quarters of the glucose having been absorbed, they found evidences of hydremia, and in the 4th hour (absorption completed) hydremia passed and hydruria came. Taken in conjunction with well known observations that direct intravenous injection of glucose may lead promptly to hydremic plethora and that glucose so injected passes with great rapidity from the blood into the tissues where it promptly ceases to exist as such, this presents the picture of a tide of glucose and water molecules moving together through the organism, the glucose finally disappearing within the cells and the water into the urine.

At the present time it might seem preferable to express the matter in terms other than those of the osmotic theory. Other-

[15] The experiment of March 27, 1912, Lusk,[12] pp. 44–45.

wise the above conception appears adequate to explain the variations of the water balance seen in connection with intravenous injections of glucose. In accordance with the solvate theory,[4] glucose molecules would tend to hydrate themselves, the number of water molecules held by a molecule of glucose depending upon the individual hydration capacity of glucose, on the amount of water present, and on the amounts and individual hydration capacities of other molecules in the solution, etc. During intravenous injections at subtolerant rates when the preponderance of all the free glucose in the body is in the blood, its hydration should lead to hydremia and this to decreased diuresis, unless an excess of water is injected with the glucose. But when glucose is injected faster than it can be chemically changed in the cells, and so passes through the renal cells and appears on the urinary side of this membrane, water must tend to accumulate there also; how much, should depend upon the quantitative distribution of glucose between the blood and urine phases, on the total supply of water in the body, etc.

This would imply that the diuretic effects of glucose are an expression of the power of the glucose molecule to hydrate itself, and of the presence of hydrated glucose on the urinary side of the renal cell. Hofmeister[16] attributed the power of salts to dehydrate colloid gels to the tendency of the salt to hydrate itself. Fischer[17] has drawn a parallel between the diuretic effects of salts and their power to dehydrate protein gels, and made experiments tending to show that the parallel holds for sugar. The above interpretation is therefore in harmony with the view previously expressed, although differing in detail.

The present results and interpretation are contrary to the generality of Allen,[18] that all the common sugars are diuretics when given intravenously, that the phenomenon of water retention during glucose administration is observed only when the sugar is given by the alimentary, subcutaneous, or other indirect route, and contrary also to his conception that antidiuresis is dependent upon the union of glucose with a colloidal substance, etc.

[16] Hofmeister, F., *Arch. exp. Path. u. Pharm.*, 1890, xxvii, 395.

[17] Fischer, M. H., Edema and Nephritis, New York, 2nd edition, 1915.

[18] Allen, F. M., Studies on Glycosuria and Diabetes, Cambridge, 1913, 1052–4.

SUMMARY AND CONCLUSIONS.

1. The technique employed in making continued injections of glucose and water into the veins of animals (rabbits, dogs) at known rates by means of a previously described volumetric pump is reported in detail, with a note on the purification of commercial glucose.

2. Experiments are cited to show that the maximum rate at which glucose can be administered continuously by vein to normal resting rabbits and dogs without causing gross glycosuria (*i.e.*, the normal intravenous glucose tolerance limit) is close to 0.85 gm. per kg. per hour in the great majority of cases, but may be found, more frequently than in man, a little higher (0.9 gm. per kg. per hour). Within rather wide limits the water administered simultaneously with the glucose may be varied without appreciably changing the rate of glucose utilization.

3. Continued intravenous injections of glucose at uniform rates between 0.9 and 2.0 gm. per kg. per hour may lead to continued excretions of glucose in the urine at uniform rates. The ratio of the intake to the output rate differs with different individuals receiving glucose at the same rate, and with different rates of injection in the same individual. When the injection rate is 1.8 to 2.0 gm. per kg. per hour, the glucose excretion per hour has represented as little as 2 and in one case as much as 10 per cent of the injection. Within rather wide limits variation of the quantity of water injected and eliminated during constant glucose injections at these rates has not been found to affect the rate of glucose utilization or excretion.

4. For any individual case the rate at which glucose enters the blood determines the rate of glucose utilization and excretion regardless of the volume of the blood and urine or the concentration of glucose in them.

5. The observed constancy of the velocities of glucose metabolism and excretion, in spite of such changes, is explained on the basis that water must so distribute itself in the body that the ratio of the plasma volume to its surface of contact with the cells at large (specific surface of the plasma phase) remains constant. The terms plasma and cells are employed in the general sense of phases in heterogeneous systems.

6. By controlling the rates of glucose and water injection it has been found possible to produce relative and absolute retardations of diuresis during glucose injections at rates causing glycosuria, as well as with those at subtolerant rates. But acceleration of the rates of water elimination (over and above that ascribable to the water given with the glucose) has not been observed without glycosuria.

7. The tendency toward water retention is attributed to the presence of free diffusible glucose in the blood and, to a less extent, in the tissues and the tendency of the glucose to hydrate itself in accordance with the solvate theory. The tendency toward diuresis, manifest only when glucose gains access to the urine, is regarded as an expression of the same process operative in another place. Where the balance of water will be found in the body during injections of glucose will depend at any moment on the quantitative distribution of glucose among the phases of the organism. Increased diuresis occurs when there is glycosuria and a sufficient supply of water in the organism.

8. Attention is called to the probable fallacy of considering the concentration of any substance in the blood plasma as a reliable index *per se* of the rate at which it enters the cells, of the rate of its utilization or excretion, or of the toxic effects produced.